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NATURAL RESISTANCE TO HELMINTHIC INFECTIONS[1,2]

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INTRODUCTION

Following the custom of recent years of choosing for this occasion a topic from one's investigational field, I have selected the subject natural resistance to helminthic infections. To follow the ramifications of this subject to their entirety would require more space and time than are available. It will be my purpose to review and analyze literature on such factors in natural resistance as diet, genetic constitution and age and to present some new data on an inhibitory nematode growth factor in duodenal mucus of older animals.

During the last decade there has been much confusion in the use of the terms resistance and immunity. In his excellent review, Taliaferro in 1929, used immunological terms to include essentially all phases of resistance. Natural resistance was considered by him an age immunity. About the same time Stoll (1929) discovered an immunity to helminthic infections to which he gave the name "self cure." Acquired resistance was used by Schwartz, Alicata and Lucker (1931), Chandler (1932a), Taylor (1934), Graham (1934), Lapage (1937) and others to designate an immunity due to previous helminthic infections.

In a review of literature from 1929 to 1938, Culbertson used immunity to cover both immunological and resistance phases whereas, in his 1941 review natural resistance and age resistance are considered apart from acquired immunity. Finally, a standard text book of bacteriology in its 1941 edition uses resistance and immunity synonymously.

In the present discussion the term natural resistance will be used to designate a host's resistance, at any age, to its helminths as developed in the absence of helminthic antigens. Natural immunity is interpreted as representing a natural incompatibility between the host and an invading

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helminth, while immunity will be used to represent the host condition that is due to helminthic antigens.

DIETARY FACTORS IN RESISTANCE TO HELMINTHS

Vitamins

In the fall of 1921 an extensive experiment was begun by myself and C. A. Herrick upon effects of the large roundworm of fowls (*Ascaridia galli*)[3] upon its host. This experiment, we have been advised and so far as we have been able to ascertain, initiated experimental helminthology in this country. It yielded an interesting by-product, namely, an experimental demonstration of marked resistance of the hosts to the development of the nematodes (Ackert and Herrick, 1928).

While this study was in progress, a survey of the intestinal helminths of 1,000 chickens in the vicinity of Manhattan, Kansas, showed that nearly half of them were infected with *Ascaridia galli* (Ackert, 1927). Inquiry into the methods of rearing showed that the fowls in the garden season were usually confined to a pen in which no green feed was available. The principal feed of kaffir or corn was inadequate and low in vitamins B and A. The years, 1924 to 1927, which were devoted to studies of the resistance of chickens to *Ascaridia* as affected by diets deficient in vitamins B and A (Zimmerman, Vincent and Ackert, 1926; Ackert, Fisher and Zimmerman, 1927) gave experimental evidence, apparently for the first time in this country, that natural resistance of animals to helminthic infection may be lowered by nutritional deficiencies. In this period, an independent study of this nature was made in the Orient by Hiraishi (1926) working with swine and ascarids.

The general plan of our experiments was to work with growing chickens of the same ages obtained from a commercial hatchery and raised under carefully controlled conditions. Such chickens were divided into the various groups, some of which were subjected to the deficient diets and others kept as controls. The same numbers, 50 to 500, infective, *Ascaridia* eggs were fed to all chickens under comparison. At the close of the experiment, the fowls were sacrificed and the worms recovered by the flushing system of Ackert and Nolf (1929). Both the numbers and the lengths of the *Ascaridia* were used as criteria of host resistance.

In our experiments the fowls on the vitamin A deficient diets[4] had markedly more and longer worms than did the control chickens (Ackert et al, 1931). Hiraishi (1926), working concurrently in Japan, fed pigs a diet deficient in vitamin A and lowered their resistance sufficiently to

[3] This nematode in the last twenty years, has been variously known as *Ascaridia perspicillum, A. lineata* and *A. galli.*

[4] White corn meal, vitamin A-free casein, yeast, vegetable fat, bone ash and salt mixture, and irradiation of fowls.

parasitize them with the human *Ascaris*. Payne, Ackert and Hartman (1925) had shown that the human *Ascaris* larvae would pass through the somatic phase of the life cycle in swine but not the intestinal phase in swine kept on an adequate diet. Clapham (1933) working on the hatching and survival of the horse ascarid, *Parascaris equorum,* in rats found vitamin A to be an important factor in resistance. Clapham's (1933) results on *Heterakis gallinae,* the cecal worm of chickens, indicated in two of three tables of data that vitamin A is a factor in the resistance of chickens to this nematode, although she did not so interpret her results. Judging from egg counts of the swine *Ascaris,* Clapham (1934) failed to obtain evidence that vitamin A deficiency increased the productivity of the ascarids. However, Wright (1935), who subjected dogs infected with ascarids to diets partially or totally deficient in vitamin A for periods of 15 to 106 days, found that the dogs on the deficient diets harbored about five times as many ascarids as did the controls on an adequate diet and exposed to the same degree of worm infection.

More critical studies on the effect of vitamin A deficiency on the resistance of rats to infection with *Trichinella spiralis* were made in 1934 by McCoy who tested the efficacy of the deficient diet by making determinations of the amount of vitamin A stored in the livers of the experimental rats. His findings supported those of previous workers with other nematodes and showed that vitamin A is an important factor in the reaction of the host to superinfections of *Trichinella spiralis*. Lawler's recent (1941) studies with the rat nematode *Strongyloides ratti* likewise showed that the absence of vitamin A lowered the rats' resistance to a primary infection and weakened the immunity to reinfection with the nematodes.

These and the other results here reviewed point strongly to the conclusion that a diet markedly deficient in vitamin A will lower the resistance of the host to its nematode parasites. The reason for this effect has not been completely elucidated. It is known that lack of vitamin A in the system affects the normal functioning of epithelial membranes of parts of the respiratory, digestive and urino-genital tracts and also those of the eyes and tear ducts. Whether the lining of the small intestine which is the habitat of most of the adult nematodes studied, is affected will require further study as will also effects on organs and tissues of the body.

As mentioned previously, early experiments on vitamin B deficiency gave evidence of lowered host resistance to the fowl *Ascaridia* (Zimmerman, Vincent and Ackert, 1926). After the multiple nature of vitamin B was established, Ackert and Nolf (1931) obtained rather striking results on numbers of worms. The lack of vitamin B which caused partial paralysis of the muscles of the digestive tract resulted in the reten-

tion of a high percentage of the worms, whereas the control chickens eliminated most of theirs.

Foster and Cort (1931) by using a diet[5] deficient in vitamins A and B and minerals lowered the resistance of dogs previously infected with the hookworm, *Ancylostoma caninum*. After the productivity of the parasites had increased markedly in the dogs on the deficient diet, the hosts were returned to an adequate diet which increased the resistance and caused an extreme inhibition of the egg production of the worms. Results from other experiments by these authors (1935) indicated that as the dogs on the deficient diet became more and more emaciated, the development and productivity of the worm parasites increased. The results of this extensive series of experiments furnish further evidence that vitamins A and B are important factors in host resistance to helminthic infections.

Among the early difficulties in the rearing of chickens in confinement was the development of a form of rickets which is caused by insufficient vitamin D. Tests on this vitamin by Ackert and Spindler in 1929 indicated that vitamin D protects the hosts against the effects of parasitism rather than by inhibiting the development of worms.

On the subject of vitamins, by the way, a struggling student wrote, "Lack of vitamin A is not as bad as lack of vitamin B which in turn will not have so many bad effects as will the lack of vitamin C and so on down the alphabet!"

Studying diet as a factor Chandler in 1932 placed rats on a diet wholly of vegetables and fruit and inferred that diet played no part in the acquisition or retention of worms so long as the host animals were not injured by the diet. But when emaciation began to develop the rats acquired more worms from a given inoculation than did the controls on more favorable rations. As this diet was deficient in vitamin B these results give additional evidence of the importance of this vitamin in natural resistance to helminths.

Other Dietary Supplements

The bare pens and cereal rations of many of the 1,000 chickens examined at Manhattan, Kansas, in the early twenties (Ackert, 1927) indicated that the fowls were being kept much of the year on rations that were almost wholly of plant origin. Preliminary tests by Ackert indicated that the inclusion of skim milk as a supplement to the diet produced chickens that were unusually resistant to the *Ascaridia*.

The studies continued by Ackert and Beach (1933) afforded evidence that a basal, cereal ration adequate for vitamins and minerals when supplemented by meat meal and by skim milk produced the most resistant

[5] Corn starch, dried ground peas, mazola oil and sodium chloride.

fowls. The cereal ration plus meat meal was second best, whereas, the cereal ration supplemented by peanut meal (a wholly plant ration), produced the least resistance in the chickens to the *Ascaridia.* These results, which were attributed to the wider range of the amino acids made available from the diets of the first two groups, and to the restricted range of amino acids in the plant diet of the chickens of the third group throw light upon the results of Chandler (1932) in which markedly more *Nippostrongylus muris* were retained in rats whose diet was wholly of plant origin (vegetables and fruit); and also upon the results of Foster and Cort (1931) whose deficient diet for dogs lacked both animal tissues and milk.

That the resistance of hosts to tapeworms is affected by diet was shown by Shorb in 1933 working with rats and the tapeworm *Hymenolepis fraterna.* A ration of white bread and water which was deficient in vitamins, minerals, proteins, and fats lowered the resistance of the rats to the tapeworms.

From the foregoing considerations, it might appear that any deficient diet that lowers the resistance of the host, will permit the helminths to increase their development and productivity. However, such was not the case with the intestinal nematode, *Ascaridia galli* as judged by its inability to grow in a host that was on a very deficient diet. In a study on the nature of the food of *Ascaridia* (*A. galli*) Ackert and Whitlock (1935) by giving chickens intra-muscular injections of a nutrient solution lacking vitamins A and B and animal proteins, lowered the resistance of the chickens without enabling the worms to thrive in the bodies of the hosts. Although many of the injected chickens died of starvation and had their resistance completely broken, fewer and shorter worms remained in them than in the controls which were kept on the regular diet (Ackert, Whitlock and Freeman, 1940).

These results indicate that there may be a critical point in the broken resistance of a host beyond which the environment becomes unfavorable for the helminth. In the present case it might be argued that the absence of nutriment in the lumen of the chicken intestine caused partial starvation of the worms, but this period of parasitism included the seven days during which the anterior ends of the worms were deeply imbedded in the mucosa where the worms doubtless fed on host substance. As Bahrs (1931) found that planaria would not grow when fed on the intestinal epithelium of starved rabbits, but would grow on that of normally fed rabbits, it is possible that the *Ascaridia* in the injected chickens were starved both from the lack of nutrient in the intestinal lumen and from the lack of food in the intestinal mucosa. At any rate, the broken resistance of the hosts did not result in increased development and productivity of their helminth parasites.

In this connection Zaiman (1940), who studied the effect of vitamin E deficiency on *Trichinella spiralis* infections in rats, found that this deficiency which may cause muscular dystrophy was less favorable for the trichinellae.

Bordering on the field of resistance as affected by diet are such studies as those of Clapham (1934a), Porter (1935b) and Otto and Landsberg (1940) on minerals, and of Reid (1940) on starvation. Clapham found that a diet markedly deficient in minerals (especially in calcium and phosphorus) favored the development of the fowl cecal worm *Heterakis gallinae*. Porter found that a diet markedly deficient in iron lowered the natural resistance of rats to the nematode *Nippostrongylus muris*. Otto and Landsberg determined that in dog hookworm disease iron therapy accompanying a deficient diet failed to affect the development or persistence of the worms, but that iron therapy may well supplement general dietary improvements. Reid found that host starvation of but 20 hours caused the removal from chickens of the complete strobila of the fowl tapeworm, *Raillietina cesticillus*.

The literature here reviewed, while not permitting a blanket statement that deficient host diets react to the benefit of the parasite, nevertheless, indicates that in general the natural resistance is lowered to helminthic infections both in the somatic and intestinal phases, when omnivorous hosts are maintained on diets deficient in vitamins A, B (complex), or D; or on rations with highly restricted sources of proteins.

GENETIC CONSTITUTION AS A FACTOR IN RESISTANCE

Epidemiological Evidence of Group Differences in Helminthiasis

That natural resistance to helminthic infections may be due in part to the genetic constitution of the host is indicated by both epidemiological data and experimental results. Miller (1908) observed that the pure-bred Zebu cattle in Trinidad resisted the ravages of strongyles better than did other breeds of cattle; he also found that sheep succumbed to the attacks of the helminths more easily than did the cattle.

Race differences in resistance to hookworms were found by Smillie and Augustine (1925), who examined nearly 2,000 rural school children. The white children had both more hookworms and a higher percentage of infection than did the negro children of the same ages living in the same communities. The authors attributed the lighter negro infections to the much greater thickness of negro epidermis.

Further evidence of racial differences in resistance to hookworms is available from the observations of Keller, Leathers and Knox (1937) made in North Carolina. Examinations of several thousand white and colored persons showed that 12.3 per cent of white persons were positive for hookworm eggs as compared with 4 per cent for the negroes of

comparable ages. These observations and others made by Keller and his associates in southern United States indicate that the resistance of negroes from five to 19 years of age to hookworm infection is markedly higher than that of whites living in the same communities.

Race differences in *Ascaris* infections were noted by Keller, Leathers and Bishop (1932) who found in a Tennessee survey that persons of the white race had heavier *Ascaris* infections than did the negroes. However, in west Tennessee, the reverse proved true. Two years later Otto and Cort (1934) reporting for the age group 5–14 years in rural areas of four southern states found *Ascaris* more prevalent and of greater intensity in the negro youths than in the whites. These authors, however, doubted that the negro race is more susceptible to *Ascaris* infection than the white race. They felt that among the negroes of those areas there was a greater prevalence of habits that were favorable for *Ascaris* infections.

Observations on differences in racial resistance to tapeworms were made by Otto (1936) who found that the whites in the southern states (five to 14 years of age) had somewhat more tapeworms (*Hymenolepis nana*) than did the negroes of the same ages. The percentages of infection were likewise constantly higher in the whites than in the negroes.

Race differences to pinworms (*Enterobius vermicularis*) were found by Cram (1940) in a study of children in nursery schools and summer camps where 33 per cent of the white children showed worm infections as compared with 19.8 per cent of the negro children. The examinations of these children together with others totaling 3,371 showed that 41.9 per cent of the whites were infected as compared with 15.5 per cent of the negroes. These results which include samplings from different parts of the country furnish further epidemiological evidence that racial or genetic constitution may be a factor in natural resistance to helminthic infections.

The epidemiological evidence of race differences in resistance of people to helminths as here reviewed seems to indicate that negroes in the southern states have greater natural protection against helminths than do the whites. This was especially true of hookworms and pinworms, but included some evidence also on *Ascaris* and the dwarf tapeworm (*Hymenolepis nana*). It is difficult to conceive of the negroes living on a higher plane either from the standpoint of nutrition or of sanitation. In the case of pinworms harbored near the nation's capital it is possible that the white nursery school children became more readily exposed to infections from the parasite eggs on the hands in games like ring-around-the-rosy than were the negro children of that region. The incidence of the various helminths probably is due to several factors, but no discussion of this subject would be complete without raising the ques-

tion of the negroes' longer association with the helminths than whites'. On the host-tolerance hypothesis it would be expected that the longer association of the negro race with the worms would result in better host-parasite adaptation and a consequent lowering of resistance to the nematode. On this basis, however, the negro infections would be larger instead of smaller. On the racial immunity hypothesis the longer association of the negroes with the worms would afford greater opportunity for the development of a grade of immunity. Whether this would become inheritable by mutations or by other means is problematical.

A desperate student who had neglected his studies once wrote in an examination, "Heredity is harmful, and should be prevented."

Experimental Evidence of Breed and Strain Differences in Resistance

The first experimental evidence of breed differences in resistance to helminthic infections appears to be from the work of Ackert, Eisenbrandt, Glading and Wilmoth in 1933 who found significant differences in the natural resistance of breeds of chickens to the large roundworm *Ascaridia galli*. Such heavy breeds and varieties as Rhode Island Reds, White Plymouth Rocks and Barred Plymouth Rocks had significantly fewer and smaller *Ascaridia* than did the lighter White Leghorn and White Minorca breeds (Ackert et al, 1935).

What appears to be the first experimental evidence of genetic differences in strains of hosts to helminthic infections was furnished by Curtis, Dunning and Bullock (1933) working with the larval tapeworm *Cysticercus fasciolaris* which sometimes caused liver tumors. The study demonstrated that some strains of rats reacted more often to the production of tumors than did others.

A strain of heavy White Minorca chickens in our studies proved to be more resistant to the *Ascaridia* than a lighter strain of the same breed with different genetic constitution (Ackert and Wilmoth, 1934). Bronze turkeys were found by Ackert and Eisenbrandt (1935) to be more resistant to *Ascaridia* of chickens than were White Leghorn fowls of the same age. Additional evidence of genetic strains within a breed was obtained by Ackert, Pratt and Freeman (1936) who, by selecting the more resistant cockerels and pullets from one flock of chickens, developed a strain of White Leghorns that was more resistant to the nematodes than were a cockerel and six hens taken at random from another flock of the same breed. Father-daughter and brother-sister matings were made in each group and all parasitized alike. The results of such tests carried on for three generations indicated that it would be possible to establish by selection a strain of chickens that is quite resistant to the *Ascaridia* as well as one that is distinctly susceptible to this nematode.

Indications of differences in breed resistance of sheep to helminthic

infections were obtained by Cameron (1935) who found that Cheviot sheep on an overcrowded pasture were more tolerant of the effects and less heavily parasitized with nematodes than were the lowland breeds such as the Border Leicester. The Shetlands and Scottish Blackface sheep were about intermediate between the other two groups. Differences in breed resistance of sheep to the nematode *Ostertagia circumcincta* were found by Stewart, Miller and Douglas in 1937 when Romney sheep were found to be significantly more resistant to the *Ostertagia* than were the Shropshires, Southdowns or Hampshires.

These data on the different degrees of sheep breed resistance to the stomach worm *Ostertagia circumcincta* were studied by a geneticist, Gregory (1937), with a view of prescribing a method of establishing within breeds, lines that are genetically resistant to the worms. The accumulating evidence pointed toward the same hereditary factors for resistance to *O. circumcincta* as to another sheep stomach worm *Haemonchus contortus* which is of paramount importance in much of the United States. Gregory's study of the data led him to think that resistant lines of sheep to stomach worms could be established without sacrificing type, conformation, quality of carcass or desirable wool characteristics.

As a step in the study of producing a resistant line of sheep to stomach worms, Gregory, Miller and Stewart (1940) tested offspring of two Hampshire rams by making fecal egg counts of trichostrongyles except *Nematodirus*. While variables of season of the year and age of the ewes were encountered, unquestioned evidence was obtained for genetic constitution as a factor. The two sires studied differed in genetic constitution for resistance and susceptibility to stomach worm infections, the offspring of one having significantly fewer worm eggs than did those of the other ram. From the results obtained, the authors felt that genetic selection should effectively change the degree of resistance or susceptibility to strongyles in populations of sheep.

The few experimental findings in the field of genetic constitution as a factor in natural resistance to helminthic infections are quite promising especially among animals that do not suffer deterioration from inbreeding as usually is the case with chickens. It is hoped that in the next decade this field may be much more fully investigated.

AGE RESISTANCE TO HELMINTHIC INFECTIONS

Experimental Evidence

The first experimental evidence of age as a factor in natural resistance to helminthic infections appears to be that of Looss (1911) who in feeding hookworm larvae to dogs observed that in young animals some of the larvae were able to reach maturity, whereas, previous investiga-

tors had failed to obtain such results in adult dogs. In 1920, Ransom and Foster working with *Ascaris* of pigs confirmed Looss's finding. The next year Ransom (1921) detected definite age resistance of chickens to the gapeworm *Syngamus trachea.*

It was at this time that Ackert and Herrick obtained experimental evidence of increased resistance with age, to the fowl ascarid of chickens (reported in 1928). A critical study of age resistance of chickens to this nematode was begun by Herrick in our laboratory in 1923–24 and concluded by him the next year in Dr. W. W. Cort's laboratory at Johns Hopkins University (Herrick, 1926). Herrick found a gradual increase in the resistance of chickens as they became older; the resistance increased with age up to 103 days after which no further increase was found in the fowls. In a 10-day period, the worms grew more than ten times as much in five-day chicks as in 103-day old fowls.

Herrick (1928) also demonstrated age resistance of dogs to hookworms. His findings were confirmed by Scott (1928) and Sarles (1929a) who also found age resistance of cats to hookworms.

That older rats develop an increased resistance against the nematode *Nippostrongylus muris* was shown by Africa (1931). Another rat parasite, the cestode *Hymenolepis fraterna* which also infects the mouse, was shown by Shorb (1933) to encounter greater resistance in older animals. Shorb found in both nursling rats and mice an initial resistance which was lost a short time after weaning.

In an extensive study of the rat nematode *Heterakis spumosa,* Winfield (1933) found an increased resistance with age of rats to this nematode. Twenty days in the life of young rats was sufficient for them to develop significantly greater resistance to the worms. Chickens develop resistance more rapidly, as shown by Ackert, Porter and Beach (1935) who found that from five- to eight-day periods in chickens about two months old may result in significant increases in resistance to growth of *Ascaridia.* Using approximately thirty-day infecting intervals Roberts (1937) obtained similar results with fowls and *Ascaridia.*

Age resistance was found by Kauzal (1934) to develop in sheep to lung worms (*Dictyocaulus filaria*) and by Roberts (1939) in cattle to gastro-intestinal helminths. Increased resistance with age was reported in 1934 by Ameel in snails to larval flukes and in 1936 by Davis in recently metamorphosed frogs to cercariae. Further evidence of host age resistance to flukes was presented by Cable (1937) experimenting with metacercariae (*Parorchis acanthus*) and gulls (*Larus argentatus*).

That tapeworms encounter more resistance in older hosts than in younger ones was shown by Ackert and Reid in 1937 working with chickens and the fowl tapeworm *Raillietina cesticillus.* The liver nematode *Capillaria hepatica* in rats and mice was shown by Luttermoser

(1938) to meet greater resistance in the older hosts, the rats showing more resistance than the mice. Rats also manifested an age resistance to *Trichinella spiralis* as shown by Nolf and Zaiman (1941). In the tests the nursling rats, however, had somewhat fewer trichinellae than the recently weaned ones.

This review of experimental work indicates that the phenomenon of increased resistance with age may occur in mammalian, avian, amphibian and molluscan hosts to trematode, cestode or to nematode helminths.

A phenomenon that may or may not be associated with age was the observation by Shorb (1933) that nursling rats gave evidence of a strong natural resistance to tapeworms and a similar finding by Nolf and Zaiman (1941) of stronger resistance in nursling rats against trichinellae than in weaned ones. It is evident from these findings that nursing was unfavorable for the development of the worms. Whether this was due to a vermicidal action of the mother's milk, to a highly restricted protein diet, or to some other factor is not known.

Epidemiological Evidence of Age Resistance

An erroneous idea crept into the literature when Smillie's (1922) epidemiological study of hookworm disease in Brazil indicated no age resistance of man to these nematodes. General works have carried the statement, and it has appeared in a review as recently as 1938. A decade passed before observations by Keller, Leathers and Ricks (1934) indicated that after 15–19 years of age, people do develop a resistance to hookworms. They found that the worm incidence fell off rather sharply after the age of 24 years. A series of studies by Keller, Leathers and their associates (Keller et al, 1937; Leathers et al, 1939) on hookworm infections in four southern states gave essentially similar results on age resistance of people to hookworm, namely a gradual increase in incidence up to 15 to 19 years of age after which there was a lower percentage and a lower intensity of hookworm infection.

In the case of the human *Ascaris,* the evidence of age resistance was positive from the first observations. The peak of infection percentages in the findings of Cort and Otto and their associates (Cort, Otto and Spindler, 1930; Otto and Cort, 1934) in the southern states occurred in the age group of seven to 14 years. The group 15 to 19 years old manifested much lower percentages of infections with these helminths. Increased resistance was found by Cram and Reardon (1939) to the pinworm *Enterobius vermicularis* in examination of 2,097 persons residing in the vicinity of Washington, D. C. Youths from six to 18 years of age had a pinworm incidence of 51 per cent as compared with 22 per cent for adults.

Clinical manifestations of schistosomiasis were found by Fisher

(1934) to be especially common in Belgian-Congo children under ten years of age but never in persons over 30 years old. The epidemiological evidence here reviewed indicates that host resistance increases with age to many species of nematodes and to some cestodes and trematodes.

ATTEMPTS TO EXPLAIN AGE RESISTANCE

Although three decades have passed since Looss found that infection experiments with parasites were more likely to succeed with young hosts, we are still trying to solve the riddle of age resistance to parasitism. Sandground (1929) in a stimulating review of host-helminth relation to age resistance and acquired immunity seriously questioned the existence of host age resistance to a helminth that is adjusted to its host. He stated, "It is now becoming evident that age resistance is not of general occurrence in helminth infections, nor is the real extent of its occurrence known." He sought to explain the occurrence of age resistance to helminths on the basis of an abnormal host hypothesis. Of the findings of Herrick (1928) and Scott (1928) on age resistance of dogs and cats to *A. caninum,* Sandground held that compared with *A. duodenale* of man, *A. caninum* is poorly adapted to both the dog and the cat. The large roundworm of fowls, *A. galli,* Sandground considered as essentially the parasite of the young chicken. He pointed to the high correlation between the exhibition of age resistance and a greater or lesser condition of abnormality in the species relation of the parasite and the host, and felt that the factors involved in age resistance could be explained by an extension of those factors required for the understanding of natural immunity.

In the same year Sarles (1929b) attributed age resistance of dogs to hookworm larvae to the action of the body defense cells since larvae penetrating the host skin caused marked local cellular reaction in old dogs but not in young ones. After Africa (1931) found evidence of age resistance of rats to *Nippostrongylus muris,* Chandler (1932b) who found somewhat less striking evidence of age resistance of rats to this parasite sought to account for the discrepancy between his work and Africa's on a host-parasite adaptation hypothesis. His larvae had been through several generations in laboratory animals whereas those of Africa had been only recently isolated. However, Graham and Porter (1934) were unable to find evidence of an adaptation of an old laboratory strain when compared with a recently isolated strain of *N. muris.*

Some support for the abnormal host hypothesis of age resistance to helminths was furnished by Porter (1935a) who studied the behavior of *Nippostrongylus muris* in rats and mice. The larvae which were obtained from a rat strain of the nematode developed more readily in rats than in mice. Porter found evidence of an age resistance in both

rats and mice to *N. muris* and observed that this increased resistance developed earlier and to a more marked degree in mice than in rats. Porter thus leaned toward the abnormal host hypothesis for the marked age resistance of the mice to the parasite but did not give reasons for the increased resistance of the older rats which presumably were the normal hosts.

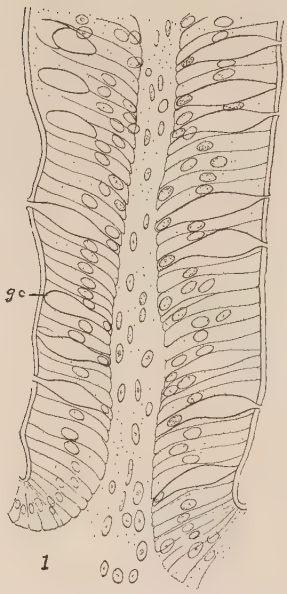
In 1935, Ackert, Porter and Beach sought to explain age resistance of chickens to *Ascaridia* on the hypothesis of a growth-inhibiting substance since Carrel and Ebeling (1921) had shown the presence of an active growth-inhibiting substance in the blood serum of chickens, and Ackert and Porter had found that bleeding lowered the resistance of the chickens to the worms (Ackert, 1925; Porter and Ackert, 1933). It was then felt that the resistance mechanism might be in the form of a fluid that passes from the intestinal wall into the lumen, or a deposit in the mucosa, or that the cellular mucosa which the young worms puncture might become tougher in the older chickens.

Foster (1936) who produced anemia in dogs by keeping them on a diet deficient in iron found a parallel between resistance and the hemoglobin level of the host. His results led him to believe that age resistance of the dogs against the hookworm *A. caninum* may be related in part tc the natural age curve of the hemoglobin level of the host.

The increased resistance of older amphibia to the penetration of cercariae of *Diplostomum flexicaudum* was attributed by Davis (1936) to the greater thickness of the skin of recently metamorphosed frogs than of large tadpoles. Davis felt that in the frogs the integumentary barrier was either too thick and horny for the cercariae to pierce, or that the mucous and poison gland secretions of the skin killed, or inactivated them.

Duodenal Mucus and Age Resistance

In a search for possible structural differences in the habitat of *Ascaridia* in younger than in older chickens, Ackert and Edgar (1938) made histological studies of portions of the duodena of such chickens. There were some differences in thickness of muscular layers, but the most striking difference was the increasing numbers of goblet cells in the chickens as they grew older (Figs. 1, 2). Studies made on chickens from two to 320 days of age showed gradual increases in the number of goblet cells per area of duodenal epithelium up to the age of 124 days. Increases corresponded closely to the manifestations of age resistance as shown by Herrick (1926). It was felt that the secretions of the goblet cells might be a factor in the elimination of the worms from the host since it had been observed repeatedly on opening the duodenum that the mucus was much more abundant in older chickens than in the younger ones. This appeared to be the first record of increased numbers of goblet



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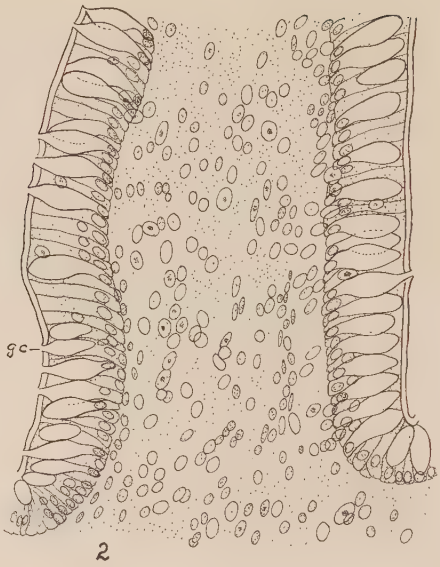

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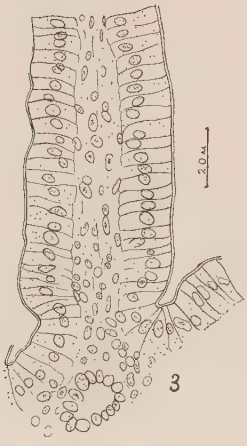

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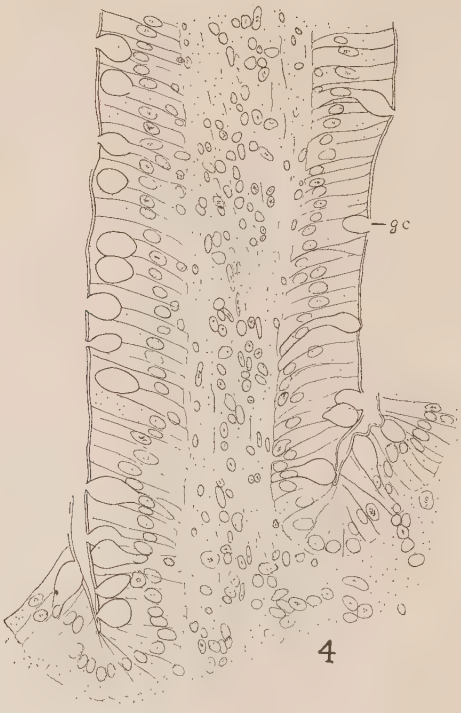

4

cells in older animals and of their possible relations to age resistance to intestinal parasitism.

Similar histological studies made on the duodenal epithelium of a number of laboratory rats of different ages showed that older rats likewise have larger numbers of duodenal goblet cells per area than do younger ones (Figs. 3, 4), but the increase is more gradual. The results, however, showed that the phenomenon of increased numbers of duodenal goblet cells is characteristic of a mammal as well as a fowl. As shown previously rats develop age resistance to their intestinal nematodes, but the resistance does not develop as rapidly in the rat as it does in the chicken. Correspondingly, the numbers of duodenal goblet cells do not increase as rapidly in the rat as they do in the chicken (Ackert, Edgar and Frick, 1939).

An Inhibitory Growth Factor in Duodenal Mucus

To test secretions of the goblet cells for an inhibitory growth factor duodenal mucus removed quickly with a blunt spatula was autoclaved and used in the ratio of 3 cc to 65 cc of the nutrient medium, that Ackert, Todd and Tanner (1938) developed for in vitro culture of *Ascaridia*. The pH of the mucus solution was adjusted to that of the control solution (approximately 6.7, as found by Ackert, 1931, for the worms' habitat in the fowl intestine). Young nematodes (*A. galli*) of the same age were removed from their normal chicken hosts, washed in warm saline solution, photographed for measurement and placed in dishes containing one of three solutions. At the end of about three days all worms were remeasured. The results of the first experiment showed that in a nonnutrient isotonic salt solution the worms lost an average of 2.5 mm. In the nutrient solution (isotonic salt-dextrose) the worms grew 11.8 mm or an increase of 18.5 per cent, whereas the worms in the medium that contained the mucus grew only 2.2 mm or an increase of 4 per cent (Table 1). The results of this test thus indicated that the mucus contained a factor that was unfavorable to the growth of the young *A. galli*.

Explanation of Figures

The figures were drawn with the aid of a camera lucida. They are all of the same magnification.

Abbreviations: *gc*, goblet cell.

Fig. 1. Median section through basal portion of a duodenal villus from a 5-day chick.

Fig. 2. Median section through basal portion of a duodenal villus from a 124-day chicken.

Fig. 3. Median section through basal portion of a duodenal villus from a 7-day rat. Note the absence of goblet cells.

Fig. 4. Median section through basal portion of a duodenal villus from a 646-day rat.

(From Ackert, Edgar and Frick, 1939.)

TABLE 1.—*Results of tests for an inhibitory nematode growth factor in duodenal mucus from chickens three months old (From Ackert, Edgar and Frick, 1939)*

Culture media	Larvae in culture (days)	Gain in length average (mm)	Per cent gain
Experiment I			
*Isotonic salt-dextrose solution plus mucus	3	2.2	4.0
Isotonic salt-dextrose solution (control)	2.5	11.8	18.5
Isotonic salt solution (no nutriment)	2	2.5 (loss)	0.0
Experiment II			
Isotonic salt-dextrose solution plus mucus	3	8.5	12.8
Isotonic salt-dextrose solution (control)	3	27.0	36.9
Isotonic salt solution (no nutriment)	3	0.2 (loss)	0.0
Experiment III			
Isotonic salt-dextrose solution plus mucus	3	2.1	2.9
Isotonic salt-dextrose solution (control)	3	14.3	19.8
Isotonic salt solution (no nutriment)	4	11.0 (loss)	0.0

* The pH of the isotonic salt-dextrose solution was 6.1; that of the autoclaved mucus was 6.0.

The results from the second and third experiments were much like those of the first, thus indicating that the autoclaved mucus from the duodenal goblet cells of chickens three months old contained a factor which retarded the growth of the *Ascaridia* in vitro.

Tests were next made with autoclaved duodenal mucus from chickens about four months (125 days) old (Table 2). The young nematodes in the nutrient medium plus mucus for three to four days either grew but little or lost size in this medium the average for the four lots being a loss of length of 4.66 per cent. On the other hand young worms in the control cultures gained an average of 12.15 per cent.

TABLE 2.—*Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 125-day chickens*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Loss in length*	Per cent gain
9M1	Iso-salt + dex. + mucus	4.5	91.50	83.50	....	8.00	....
9M2	Iso-salt + dex. + mucus	3.0	61.50	64.00	2.50	...	4.06
9M3	Iso-salt + dex. + mucus	3.5	58.98	57.00	....	1.98	....
9M4	Iso-salt + dex. + mucus	4.5	97.98	87.50	....	10.48	....
						Av. loss	4.66
	(Control)						
9D1	Iso-salt + dex.	3.0	58.98	66.00	7.02	...	11.9
9D2	Iso-salt + dex.	4.5	88.98	100.00	11.02	...	12.4
						Av.	12.15

* Per cent determined, but not here recorded.

The next tests were made on mucus from chickens about two months (66 days) of age. The mucus was autoclaved as usual and introduced at the ratio of 3 cc to 65 cc of nutrient medium. The young worms in six such cultures from one to three days made in each case a substantial gain in length, an average of 30.58 per cent. The controls gained but little more, an average of 40.75 per cent (Table 3). These results indicated that while there was some retardation of the growth of the worms, the inhibiting factor in the two-month mucus was not as potent as in the mucus from the 125-day chickens.

TABLE 3.—*Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 66-day chickens*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Per cent gain
3M1	Iso-salt + dex. + mucus	3	48.75	67.00	18.25	37.4
3M2	Iso-salt + dex. + mucus	2	37.50	53.66	16.16	43.0
3M3	Iso-salt + dex. + mucus	3	52.50	75.00	22.50	42.8
3M4	Iso-salt + dex. + mucus	1	52.50	59.50	7.00	13.3
3M5	Iso-salt + dex. + mucus	2	30.83	38.50	7.67	24.8
3M6	Iso-salt + dex. + mucus	3	43.75	53.50	9.75	22.2
						Av. 30.58
	(Control)					
3DC1	Iso-salt + dex.	2	35.00	55.50	20.50	58.5
3DC2	Iso-salt + dex.	1	48.75	60.00	11.25	23.0
						Av. 40.75

The question arose as to whether the substance responsible for the inhibitions of the growth of the nematodes was soluble. Accordingly, the duodenal mucus from chickens 171 days old after being autoclaved was ground with sterile sand and a 0.9 per cent saline extract prepared. This was used in three nutrient cultures in the place of mucus; also in four other cultures, washings from autoclaved mucus were added to the nutrient media; four nutrient control cultures were prepared. After being in these respective cultures for from six to nine days, it was found that in the nutrient plus mucus extract cultures one group had gained while two had lost in length giving an average loss of 7.2 per cent. In the nutrient plus mucus washings cultures one group gained while three lost in length giving an average loss of 1.79 per cent. The worms in the control cultures all increased in length, gaining an average of 9.25 per cent (Table 4). The results from this test indicated that the growth-retarding factor in the autoclaved mucus was soluble to some extent in 0.9 per cent saline. Subsequent tests made on the above washings indicated that the inhibitory factor was capable of passing through a Berkefeld filter. That the effect of the inhibitory growth factor did not produce permanent effects upon the worms was shown in a subsequent test in which the worms of cultures 11G1 and 11G3 (Table 4) on being

TABLE 4.—*Test for inhibitory nematode growth factor in duodenal mucus (autoclaved) from 171-day chickens*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain in length	Loss in length*	Per cent gain
11G1	Iso-salt + dex. + ground mucus extract	8	66.30	59.50	....	6.80	....
11G3	Iso-salt + dex. + ground mucus extract	8	73.10	59.50	....	13.60	....
11G4	Iso-salt + dex. + ground mucus extract	6.5	57.80	63.50	5.70	....	9.85
						Av. loss	7.23
11W1	Iso-salt + dex. + mucus washings	9	76.50	89.25	12.75	....	16.60
11W2	Iso-salt + dex. + mucus washings	8	73.10	67.66	....	5.44	....
11W3	Iso-salt + dex. + mucus washings	9	64.60	62.50	....	2.10	....
11W4	Iso-salt + dex. + mucus washings	8	73.64	64.00	....	9.64	....
						Av. loss	1.79
	(Control)						
11D1	Iso-salt + dex.	6	66.30	77.00	10.70	....	16.14
11D2	Iso-salt + dex.	6	56.10	60.00	3.90	....	6.95
11D3	Iso-salt + dex.	6.5	68.00	75.00	7.00	....	10.29
11D4	Iso-salt + dex.	6	71.40	74.00	2.60	....	3.64
						Av.	9.25

* Per cent determined, but not here recorded.

placed in fresh nutrient media made an average gain of 32.7 per cent in six days.

In a series of tests young *A. galli* were cultured in media to which was added autoclaved duodenal mucus from chickens arranged in a graded series from susceptible to resistant hosts ranging in ages from 45 to 125 days (Table 5).

Worms subjected to 45-day mucus made as much gain as did the controls indicating no effect of an inhibiting factor. The worms subjected to fowl mucus 54 days old gave evidence of some retardation of growth, 17.9 mm as compared with 21.7 mm for the control worms. Gradual increases in retardation of the growth of the worms were demonstrated with fowl mucus up to 70 days old. When 107-day mucus was used, the

TABLE 5.—*Showing reactions of* A. galli *cultured in media to which was added autoclaved duodenal mucus from chickens arranged in a graded series from susceptible to resistant hosts*

Worms group	Age of mucus (days)	Experimental worms		Controls
		Av. gain (mm)	Av. loss (mm)	Av. gain (mm)
A	45	9.598	...	9.205
B	54	17.916	...	21.740
C	63	14.406	...	17.090
D	66	13.555	...	15.880
E	70	12.090	...	42.380
F	107	4.750	...	28.530
G	117	.....	0.83	8.250
H	125	.....	4.19	9.020

results indicated a marked increase in inhibiting power as the experimental worms gained an average of 4.7 mm as compared with 28.5 mm for the controls. The mucus from chickens 117 and 125 days old, respectively, showed a strong retarding effect by causing the worms to lose length while the controls were gaining 8.2 and 9.0 mm, respectively.

While the results in these various tests were indicative of a more potent growth inhibiting factor in the duodenal mucus of older chickens than in younger ones, the numbers of worms were not sufficient for statistical treatment. It was therefore determined to arrange for an extensive series of tests which would give comparisons of worms of different ages subjected to mucus from chickens of various ages. In these tests the duodenal mucus was not autoclaved; in other respects the tests were essentially similar to the previous ones, namely, that the mucus was introduced in the ratio of 3 cc to 65 cc of nutrient medium and all cultures were adjusted to the same pH of approximately 6.7. *A. galli* from 30 to 90 days of age taken by 10-day intervals were treated with mucus from chickens 60 to 140 days of age considered by 20-day intervals.

The data in this series of experiments when treated by analysis of variance[6] yielded the highly significant F value of 6.35. It has thus been demonstrated that the duodenal mucus samples differed significantly in the degree of nematode growth inhibition in vitro, and that the effectiveness of the mucus as an inhibitor varied directly with the age of the chickens from which the mucus was taken (Frick and Ackert, 1941). Complete details of these results and of the statistical studies are given by Frick (1941).

Since this substance is present in mucus that has been autoclaved at temperatures that ordinarily destroy antibodies, the explanation of increased natural resistance with age to intestinal worms does not lend itself to an antigen-antibody hypothesis. The host body through its intestinal goblet cells appears to elaborate a thermo-stable substance that is harmful to the helminths.

In tests recently completed by Eisenbrandt and Ackert (1941), it was found that duodenal mucus extracts of adult dogs and hogs caused the early death (24 to 72 hours) of all fowl nematodes (*A. galli*) tested in vitro; whereas, worms in control nutrient solutions lived for a week.

SUMMARY

Evidence from experimental results and epidemiological data indicates that the degree of natural resistance of a host to its helminth parasites is dependent largely upon its diet, its genetic constitution and its age. Diets of man and other omnivorous animals, to serve in developing

[6] Indebtedness is acknowledged to Dr. H. C. Fryer, Department of Mathematics, Kansas State College, Manhattan, Kansas, for counsel on the statistical treatment of data.

the most potent resistance, should include in the balanced ration, an adequate amount of vitamins A, B (complex), and D, suitable minerals and a range of proteins as supplied in cereals, meat, and milk. Such a diet taken from the young to the adult condition will ordinarily result in the development of a high natural resistance to helminthic infections. By selection it appears to be possible to alter the genetic constitutions in succeeding generations of animals in such manner as to increase their natural resistance to parasites. Just what the nature of the natural resistance is has not been completely elucidated; but there are indications that duodenal mucus from goblet cells contains a thermostable substance that inhibits the development of intestinal helminths.

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FIVE NEW SPECIES OF AVIAN SCHISTOSOMES FROM WISCONSIN AND MICHIGAN WITH THE LIFE CYCLE OF *GIGANTOBILHARZIA GYRAULI* (BRACKETT, 1940)*

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In a recent summary of the distribution of schistosome dermatitis in this country (Brackett, 1941), it was reported that the disease is to be found chiefly in the northern parts of Michigan, Wisconsin, Minnesota, and Manitoba. In this area, *Cercaria stagnicolae* Talbot, 1936, is responsible for the largest proportion of the outbreaks. This can partially be explained by the fact that its snail hosts, varieties of *Stagnicola emarginata,* prefer a habitat that is also chosen by bathers and that this snail species reaches the height of its development during the resorting season. Within this important center of schistosome dermatitis, the disease was found to be distributed unequally and to fluctuate from year to year. Neither of these features could be completely correlated with the presence or absence or fluctuations of the snail hosts and it was suggested that the explanation probably would be found when the life cycle of *C. stagnicolae* is demonstrated and the adult stage and its host become known. Talbot (1936) and others have attempted to determine the life cycle of *C. stagnicolae* by exposing birds and mammals to this form. Perhaps these experiments have all been unsuccessful because this cercaria may be very specific in its definitive host and the proper bird or mammal might not have been used. In an effort to become better acquainted with the adult schistosome fauna of the dermatitis areas McLeod (1940) and the author (Brackett, 1940a) and others have examined many birds and some mammals from Manitoba, Wisconsin, and Michigan specifically for blood flukes. The results of these investigations combined with reports in the literature showed that about 10 species of schistosomes occurred in birds to be found in Wisconsin and vicinity. The present paper reports the results of the examination of birds in the spring and summer of 1940 in Wisconsin and Michigan during which time 5 previously undescribed schistosomes were found.

The principal difficulty in the taxonomy of the avian schistosomes is that many of the known forms are described incompletely due to the in-

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adequacy of the material. There is probably no trematode group in which it is more difficult to get suitable specimens for study. The avian schistosomes are often very minute and extremely fragile and, therefore, practically impossible to extricate if they are in the smaller veins overlying the intestine. In addition, since the schistosomes are diecious, it is necessary to obtain specimens of both sexes for a complete specific description. This has often not been possible. Because the descriptions of the species have so frequently been incomplete, and because there are unquestionably many species yet unknown, it is obviously difficult to define and delimit the genera of the family SCHISTOSOMATIDAE. In view of these facts the species to be described in this paper are placed in existing genera with no effort made to modify the generic conceptions. It is believed that any further attempt to revise the schistosome genera should be withheld until the species have been more clearly defined and until the group is more completely known.

In contrast to the conservatism regarding the genera the author has tended to go to extremes in splitting species. The principal reason for this is to avoid any chance of building up a species complex which might later complicate the solution of the life cycles of these forms. Talbot, by very careful and painstaking studies, cleared up a species complex of schistosome cercariae by demonstrating that what had been considered a single species, *C. elvae* Miller, 1923, actually represented three species very similar in morphology. There is no reason to believe that the adults of these cercariae should not likewise be similar and easily confused. If life cycle experiments show that more than one of the species of adults herein described develop from a single species of cercaria, it will cause very little confusion to drop some of the forms into synonomy. On the other hand if several similar adults were thrown together as a single species, it would only be with great difficulty that they could later be conclusively separated.

METHODS

In all cases the birds were either shot or trapped by the author. Because it is desirable to make the examinations as soon as possible after the death of the host many of them were examined in the field. This is difficult but so important that it is well worth the effort required to get the necessary apparatus into the field. Ordinarily a make-shift laboratory was set up in an automobile and the car driven as close to the collecting areas as feasible. If the worms were found in some of the larger veins, these vessels were tied off and removed to saline before being opened. If the worms were in the smaller mesenteric veins, the mesenteries were stripped from the organs and placed in saline on a glass plate. With fine instruments the worms were then dissected from the veins. At any event the cloaca and large intestine and other sections of the intestine were split and

laid out on a glass plate so that the vessels on their surfaces could be examined with the dissecting microscope. This is a very important step in detecting the smaller forms or females and no examination is complete without this procedure. Worms seen in the minute veins on the intestinal wall were best recovered by cutting across the vessel close to the specimen and then gently forcing the worms out by lightly stroking the vessel with a fine but blunt instrument. The worms were transferred to saline and then dropped into Gilson's fixative at 60° C. In most cases, the worms relaxed reasonably well with this treatment although the filamentous forms never completely straightened out. Staining results varied with each species but alum-cochineal yielded the most consistently good specimens. Semichon's carmine frequently gave good results.

The Genus *Pseudobilharziella*

Some very minute blood flukes were found in four of the species of ducks examined in Wisconsin. These worms were all in the small veins on the surface of the large intestine and cloaca. Examination of stained specimens as well as observations on living specimens showed that all of these worms are very similar in morphology but it is possible to separate them into four forms which are here described as separate species. These worms are closely related and fit readily into the genus *Pseudobilharziella* as defined by Ejsmont (1929) since they are filamentous, have both oral and ventral suckers well developed, have a short but distinct gynaecophoric canal, and have the anterior part of the body much shorter and somewhat wider than the long posterior part which contains the numerous testes.

Pseudobilharziella waubesensis n. sp.

(Figs. 1–2)

Specific description: Pseudobilharziella: Filamentous, cylindrical worms up to 5.62 mm in length. Both sexes reasonably similar in size and shape but female slightly wider than the section of the male behind the gynaecophoric canal. Width of body of female uniformally about 0.047 mm. Width of male about 0.047 to 0.062 mm at acetabulum, from 0.072 to 0.082 mm at gynaecophoric canal, and about 0.032 mm behind the gynaecophoric canal. Both oral and ventral suckers well developed.

Male: Acetabulum about 0.040 mm in diameter and about 0.275 mm from the anterior end and with several circles of fine spines. Body finely striated but not spined. Gynaecophoric canal well defined, 0.252 to 0.306 mm in length and about 0.540 mm from anterior end and thickly spined. Testes approximately 100 in number, about 0.035 mm long and 0.018 mm wide and arranged in tandem. Testes separated by a distance equal to or greater than the length of a testis; the body constricts between them to give a beaded appearance to the whole section back of the gynaecophoric canal. Testes extend from posterior end of the gynaecophoric canal to the end of the body. Vas deferens leads forward as a fine tubule to a point just back of the acetabulum where it turns posteriorly and expands into a large seminal vesicle which is coiled into about ten loops. The sperm duct leads directly as a simple tubule to the genital pore at the anterior end of the gynaecophoric canal. Oral sucker about 0.040 mm long and 0.028 mm wide. Mouth slightly subterminal. Simple esophagus bifurcates just cephalad of the acetabulum. Cecal reunion not observed.

Female: Acetabulum 0.306 mm from anterior end. Ovary not coiled, only slightly irregular in its course; about 0.325 mm long and situated 0.684 mm from anterior end of body. Ovary opens almost directly into the seminal receptacle which is about 0.050 mm long. Oviduct arises at posterior end of seminal receptacle, immediately turns anteriorly and proceeds as a small tubule to a point about mid-way between the acetabulum and the anterior tip of the ovary where it expands into the large thin walled oötype. A spindle shaped egg about 0.150 mm in length was enclosed in the oötype of the specimen examined. Its terminal spines extended in either direction beyond the limits of the main body of the oötype. The uterus leads forward from the oötype past the acetabulum but the exact location of the genital pore remains undetermined. The vitellaria consist of a great number of small follicles which start from a point about 0.150 mm caudad of the ovary and lie on both sides of the cecum, extending presumably to the posterior end of the body. Vitelline duct not observed. Simple esophagus bifurcates just cephalad of the acetabulum and the ceca reunite again in the region of the seminal receptacle.

Hosts: Nyroca collaris (Donovan) and *Mareca americana* (Gmelin).

Location: Intestinal and cloacal veins.

Locality: Lake Waubesa, near Madison, Wis., U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 44868.

This description is based on detailed studies of 3 complete or nearly complete male specimens and 1 nearly complete female specimen from a baldpate duck, and 1 nearly complete male specimen from a ring-necked duck.

Pseudobilharziella kegonsensis n. sp.

(Fig. 3)

Specific description: Pseudobilharziella: Male: Long, cylindrical body of uniform diameter except for the slightly enlarged region of the gynaecophoric canal and the expanded posterior tip. Body length about 4.03 mm and maximum body width (excluding gynaecophoric canal region) 0.054 mm. Body surface finely striated but not spined. Oral sucker 0.036 mm long and 0.025 mm wide. Ventral sucker (0.288 mm from anterior end) 0.036 mm in diameter, may protrude noticeably, and bears a number of circles of fine spines. Gynaecophoric canal well defined, 0.144 mm long and situated 0.54 mm from anterior end, and thickly spined. Body in this region 0.65 mm in diameter. Testes about 150 in number, 0.022 mm long and 0.032 mm wide, arranged in tandem and lying close together but usually not contiguous. They extend from posterior margin of the gynaecophoric canal to the posterior tip of the body. The vas deferens leads forward as a fine tubule to the region of the acetabulum where it turns posteriorly and expands into the seminal vesicle. The seminal vesicle is coiled into about 6 loops and extends almost to the gynaecophoric canal. At its posterior end it narrows into a simple, short sperm duct that empties through the genital pore which is on a small papilla at the anterior end of the gynaecophoric canal. The oral opening is slightly ventral in position. The simple esophagus bifurcates into the intestinal ceca about mid-way between the oral and ventral suckers. The point of cecal reunion was not determined.

Female: Unknown. Eggs in intestinal mucosa of host were spindle-shaped.

Host: Nyroca valisineria (Wilson).

Location: Cloacal vein.

Locality: Lake Kegonsa, near Madison, Wis., U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44865.

This description is based on a detailed study of a single, complete male specimen stained with Semichon's carmine stain. Several worms were seen at autopsy of the one infected canvas-back duck but it was possible

to recover only one of them. The mucosa of the large intestine and cloaca of this bird contained numerous spindle shaped eggs. These hatched within a few minutes after being placed in tap water and the miracidia proved to be typical of the family SCHISTOSOMATIDAE. The miracidia are about 0.20 mm in length.

Pseudobilharziella horiconensis n. sp.

(Figs. 4a and 6)

Specific description: Pseudobilharziella: Male: Filamentous body about 3.56 mm long which may be divided into three parts: a short section from the anterior end to the gynaecophoric canal which is about 0.057 mm wide; the anterior half of the section back of the gynaecophoric canal which has roughly parallel margins and is about 0.038 mm wide; and the posterior section of the body which is very narrow and constricts between the testes giving a beaded appearance. Body surface finely striated but not spined. Oral sucker 0.036 mm long and 0.032 mm in diameter and possessing several circles of fine spines. Gynaecophoric canal well defined, 0.125 mm long and situated 0.432 mm from anterior end and thickly spined. Body in this region is 0.065 mm wide. Testes not over 115 in number; extend from posterior end of gynaecophoric canal to posterior end of body in tandem arrangement. Those in the anterior section of this body region are so closely arranged they are contiguous while those in the posterior, narrower section of the body may be a considerable distance from each other with the body wall constricting between them. The vas deferens leads forward as a fine tubule to a point just behind the acetabulum where it bends posteriorly and expands into the wide coiled seminal vesicle. The seminal vesicle is coiled into about four to six loops and extends almost to the gynaecophoric canal. At its posterior end it narrows into a simple, short sperm duct that empties through the genital pore which is on a small papilla at the anterior end of the gynaecophoric canal and slightly to the right of the mid-line. The oral opening is terminal or slightly sub-terminal. The simple esophagus bifurcates closer to the ventral than to the oral sucker. The location of the cecal reunion was not determined.

Female: Unknown.

Host: Nyroca americana (Eyton).

Location: Cloacal vein.

Locality: Horicon Marsh, near Mayville, Wis., U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44866.

This description is based on a detailed study of a single, complete male specimen stained with Semichon's stain.

Pseudobilharziella burnetti n. sp.

(Fig. 5)

Specific description: Pseudobilharziella: Male: Long, cylindrical body of uniform diameter except for the slightly enlarged region of the gynaecophoric canal. Posterior tip not seen. Length of body not determined; body width about 0.048 mm. Body surface finely striated but not spined. Oral sucker wider than long; 0.032 mm long and 0.036 mm wide. Ventral sucker about 0.216 mm from anterior end and 0.040 mm in diameter, and has several circles of fine spines. Gynaecophoric canal well defined; 0.144 mm long and situated 0.45 mm from anterior end, and is thickly spined. Body in this region 0.065 mm in diameter. Number of testes not determined. Testes wider than long; arranged in tandem and contiguous with each other; extending from the posterior margin of the gynaecophoric canal presumably to the caudal end of the body. The vas deferens leads forward as a fine tubule to a point mid-way between the acetabulum and the gynaecophoric canal where it bends

posteriorly and expands into a short, wide seminal vesicle which is coiled in three to four loops. At its posterior end the seminal vesicle narrows into a simple, short sperm duct that empties through the genital pore on the right side of the anterior end of the gynaecophoric canal. The oral opening is sub-terminal. The simple esophagus bifurcates into the intestinal ceca just in front of the acetabulum. The point of cecal reunion was not determined.

Female: Unknown.
Host: Nyroca collaris (Donovan).
Location: Cloacal vein.
Locality: Horicon Marsh near Burnett, Wis., U. S. A.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44867.

The description is based on a detailed study of a single, male specimen in two fragments stained with Semichon's carmine stain.

These new species are differentiated one from the other as well as from previously described members of the genus in the following key. This key is based only on the males because, with the exception of *P. waubesensis,* the females are either unknown or inadequately described. Apparently the males of this group are more apt to be found.

Key to Males of the Genus *Pseudobilharziella*

1. Testes on each side of common cecum; cecum usually convoluted . . 2
   Testes arranged linearally in tandem; common cecum more or less straight 4
2. Testes numerous, more than one in each cross section 3
   Usually only one testis in each cross section; these lie alternately on each side of cecum . . . . *Pseudobilharziella yokogawai* (Oiso, 1927)
3. Less than 4 mm in length; genital pore anterior to cecal union.
   Pseudobilharziella querquedulae McLeod, 1937
   More than 5 mm in length; genital pore posterior to cecal union.
   Pseudobilharziella kowalewskii Ejsmont, 1929
4. Larger worms, 11–12 mm in length and 0.15 mm in width; testes large, 0.08 mm by 0.06 mm.
   Pseudobilharziella filiformis Szidat, 1939
   Smaller worms, less than 5–6 mm in length and very slender 5
5. Body cylindrical and uniform in diameter 6
   All or part of body back of gynaecophoric canal constricted between testes to give a beaded appearance 7
6. Seminal vesicle only one-half the length of the distance from the acetabulum to gynaecophoric canal.
   Pseudobilharziella burnetti, n. sp.
   Seminal vesicle long, stretching from acetabulum to gynaecophoric canal . . . . . *Pseudobilharziella kegonsensis* n. sp.
7. Gynaecophoric canal over 0.25 mm long; seminal vesicle coiled in about 10 loops, extending from acetabulum to gynaecophoric canal.
   Pseudobilharziella waubesensis n. sp.

Gynaecophoric canal about 0.150 mm long; seminal vesicle wide and forming only 4–6 loops .... *Pseudobilharziella horiconensis,* n. sp.

If the new species described here are not in the future proved to constitute a single species, then the genus *Pseudobilharziella* rivals the genus *Schistosoma* in size. The eight species now placed in the genus *Pseudobilharziella* seem to form a rather homogeneous group as far as can be determined from the yet inadequate material available. In addition they are all found in ducks with the exception of *P. filiformis* which occurs in the swan. Males of all eight species have been described. In *P. querquedulae* and *P. filiformis* a cirrus sac and related organs and glands are reported. No reference is made to these structures in *P. kowalewskii* or *P. yokogawai.* Although these structures were not seen in the new species even after careful study, it is still possible that they might be present. It is the author's opinion that a cirrus and cirrus sac would be found to exist if more and better prepared specimens were available. In the males of all eight species the testes are numerous, completely filling the body caudad of the gynaecophoric canal; a seminal vesicle is prominent, loosely coiled and generally fills the region between the intestinal ceca; the genital pore is situated at the anterior end of the gynaecophoric canal; and the gynaecophoric canal is short but prominent, situated near the anterior end of the body and set with numerous fine spines.

In the literature the females of *P. filiformis* and *P. yokogawai* have been reported. Those of the former species are smaller than the males while those of the latter species are longer although more slender than the males. Nothing concerning the structure of these females is available. The author has seen females of *P. querquedulae* but has been unable to obtain specimens suitable for study. All that can be said is that they are much smaller than the males. Thus it is seen that the description of the female of *P. waubesensis* adds considerably to knowledge of the genus *Pseudobilharziella.* The sexes of this species are similar in general size and appearance. The ovary is in the anterior end of the worm. It opens into a fairly large seminal receptacle at its posterior end which in turn opens into the oviduct leading forward from this point. About mid-way between the ovary and the acetabulum, the oviduct expands into a large thin-walled oötype. The uterus leads forward past the acetabulum but the location of the genital pore is not determined. The vitellaria consist of numerous small follicles that completely fill the body back of the seminal receptacle.

The eggs of *P. filiformis* and *P. yokogawai* have been described in the literature and those of *P. waubesensis, P. kegonsensis,* and *P. querquedulae* have been seen by the author. In all cases except of *P. filiformis* the eggs are spindle-shaped with a long blunt spine on either end. The

egg of *P. filiformis* is semi-oval with only a very tiny spine at one end. In all cases the eggs contain fully developed miracidia when passed from the host and these hatch within a few minutes after reaching water.

The life cycle of *P. yokogawai* has been described by Oiso (1927). He found in Formosa that cercariae very similar to the typical dermatitis-producing schistosome cercariae of North America would penetrate the skin of ducks and develop in about two weeks into mature schistosomes in the small veins of the lower gut. Miracidia which hatched from the spindle shaped eggs of this species penetrated into lymnaeid snails. No life cycles of other members of the genus have been demonstrated experimentally but upon epidemiological evidence the author (Brackett, 1940c) suggested that the larval stage of *P. querquedulae* may prove to be *C. physellae* which develops in *Physa* snails in Wisconsin and Michigan. It seems not unlikely then that subsequent experiments will show that some of the new species of *Pseudobilharziella* described here develop from some of the cercariae involved in outbreaks of dermatitis.

The Genus *Gigantobilharzia*

In certain restricted regions in Wisconsin a large percentage of the red-winged and yellow-headed blackbirds were found to harbor a long filamentous blood fluke in the tiny veins encircling the small intestine. The intestines of these worms often were packed with dark material which made them quite conspicuous especially under magnification. While large numbers of these worms were seen, very few specimens suitable for study could be removed since their great length prevented their being pushed or pulled out of the vessels.

These worms have been placed in the genus *Gigantobilharzia* and since, as will be presently described, the miracidia were shown after development in snails to give rise to *Cercaria gyrauli* Brackett, 1940, this species then becomes *Gigantobilharzia gyrauli* (Brackett, 1940) n. comb.

Gigantobilharzia gyrauli (Brackett, 1940)

(Figs. 6–7)

Specific description: Gigantobilharzia: Extremely filamentous. Sexes similar in size and shape. Body cylindrical, about 10 mm in length with posterior tip bluntly rounded. Body width uniformly about 0.045 to 0.050 mm. Oral sucker well developed and 0.030 mm long. Ventral sucker absent.

Male: Gynaecophoric canal present; 0.8 mm in length and 1.38 mm from anterior tip of body. Body 0.054 mm wide at gynaecophoric canal. Testes very numerous; distance between each testis equal to or greater than diameter of each follicle. They extend from a point about 0.26 mm back of gynaecophoric canal to posterior tip of body. Vas deferens leads forward from testes as a very fine tubule to a point about 0.28 mm from anterior end of body where it bends posteriorly and expands into the seminal vesicle. Seminal vesicle about 0.26 mm long and coiled in 8 to 10 loops. Sperm duct leads directly as a plain tubule to the genital pore which is situated on a small papilla at anterior end of gynaecophoric canal.

Female: Ovary coiled in about 10 loops; 0.45 mm in length extending posteriorly

from a point 1.4 mm from anterior tip of body. Posterior end of ovary narrows into a tubule 0.018 mm in length which leads into a seminal receptacle 0.15 mm in length. Oviduct arises at posterior end of seminal receptacle and immediately turns anteriorly and continues to a point 1.115 mm from anterior end of body where it expands into a large thin walled oötype. The uterus leads forward from the oötype to the genital pore which is situated immediately behind the oral sucker. Vitellaria in numerous follicles closely arranged and practically filling the entire body from a point a short distance caudad of the seminal receptacle to the posterior tip of the body. Vitelline duct leads forward and joins the oviduct just before it enters the oötype. No eggs observed *in utero*. Eggs only slightly elongate, about 0.060 mm long, thin shelled and without spine. Esophagus bifurcates 0.54 mm from anterior end and ceca reunite just caudad of seminal receptacle.

Hosts: Agelaius phoeniceus arctolegus Oberholser and *Xanthocephalus xanthocephalus* (Bonaparte).

Location: Intestinal veins.

Locality: Sun Prairie, Wis., U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 44864.

The description of this species is based on detailed studies of fragments of four males and four females and general observations on many more fragments. The worms were collected from 20 red-winged and 5 yellow-headed blackbirds.

This species differs from *G. acotylae* Odhner, 1910 in being much smaller, in having a better developed oral sucker, and in having a longer gynaecophoric canal situated farther back from the anterior end. It is unlike both *G. egreta* Lal, 1937 and *G. monocotylea* Szidat, 1930 since neither of these possess a gynaecophoric canal.

Herring and ring-billed gulls in the region of the Biological Station of the University of Michigan at Douglas Lake, Michigan, were found to harbor *Microbilharzia lari* (McLeod, 1937). In addition some of these birds were found to harbor what proved to be an undescribed form which has also been placed in the genus *Gigantobilharzia*. These worms are reasonably large and were most frequently found in the larger intestinal veins and hepatic portal vein.

Gigantobilharzia lawayi n. sp.

(Figs. 8–9)

Specific description: Gigantobilharzia: Extremely filamentous. Female as long as or longer than male and similar in shape but more slender.

Male: About 7.3 mm in length and 0.150 mm in diameter at gynaecophoric canal and 0.105 mm elsewhere. Gynaecophoric canal present, anterior end distinct but posterior end terminates obscurely; 0.63 to 0.85 mm from anterior end and may be as long as 2.25 mm or may be shorter. Testes over 500 in number, wider than long, and arranged in tandem and usually close together. Testes extend from posterior tip of body to a point further forward than the posterior end of gynaecophoric canal but not beyond its equator. Vas deferens leads forward as a fine tubule to a point from 0.37 to 0.52 mm from the anterior end where it bends posteriorly and expands into the seminal vesicle. Seminal vesicle 0.30 to 0.37 mm in length and coiled in about six or seven loops. Cirrus and cirrus sac present. Genital pore situated at anterior end of gynaecophoric canal. Intestine bifurcates from 0.22 to 0.30 mm from anterior end. Point of cecal reunion not seen.

Female: About 10 mm in length and a maximum width of 0.060 mm. Ovary coiled in 8 to 10 loops; 0.22 mm in length and situated 0.71 mm from anterior end.

Posterior end of ovary opens almost directly into seminal receptacle which is 0.075 mm in length. Oviduct arises at posterior end of seminal receptacle and immediately turns anteriorly and proceeds to a point 0.60 mm from anterior end where it expands into a large thin-walled oötype. The uterus runs forward from the oötype and the genital pore is probably just behind the oral opening. Vitellaria small and numerous and start a short distance posterior to the seminal receptacle and fill the body caudad of this point. Vitelline duct not observed. No eggs seen in the uterus of the worms or in the intestinal mucosa of the hosts. Digestive system not observed.

Hosts: *Larus argentatus smithsonianus* Coues and *L. delawarensis* Ord.
Location: Intestinal veins and hepatic portal.
Locality: Douglas Lake, Michigan, U. S. A.
Type specimens: U. S. Nat. Mus. Helm. Coll. Nos. 44862 and 44863.

The description of this species is based on detailed studies of 4 entire or almost entire male specimens and one almost entire female. Reference to the following key to the males of the genus *Gigantobilharzia* will serve to differentiate this species from others of the genus.

Key to Males of the Genus *Gigantobilharzia*

1. Gynaecophoric canal present, more or less well developed 2
   Gynaecophoric canal absent 4
2. Oral sucker present 3
   Oral sucker absent ..... *Gigantobilharzia lawayi* n. sp.
3. 140 to 165 mm long and 0.25 to 0.35 mm wide.
   Gigantobilharzia acotylea Odhner, 1910
   About 10 mm long and 0.06 mm wide.
   Gigantobilharzia gyrauli (Brackett, 1940)
4. Oral sucker present .... *Gigantobilharzia monocotylea* Szidat, 1930
   Oral sucker absent ..... *Gigantobilharzia egreta* Lal, 1937

The genus *Gigantobilharzia* is difficult to define precisely because the species now referred to it may not be cogeneric; but because the group is not yet well enough known a revised generic diagnosis is not proposed even though Odhner's (1910) characterization of the genus is now inadequate. However, it seems worth while to call attention to several points. None of the species possess an acetabulum although an oral sucker may or may not be present. A gynaecophoric canal is absent in some species and poorly defined in others. The testes, at least in some species (*G. lawayi* and *G. egreta*), are more numerous than in any other known forms. The eggs are known for 3 species and all are oval without spines or with only a tiny spine at one end. In addition, as contrasted with the genus *Pseudobilharziella,* these species are not found in ducks but in gulls, blackbirds and the cattle egret of India.

The Life Cycle of *Gigantobilharzia gyrauli*

Usually the mucosa of the intestine of infected blackbirds was found to contain large numbers of the eggs of *G. gyrauli.* Many of these eggs

contained fully developed miracidia and it is assumed that they are in this condition when passed from the host. Eggs scraped from the mucosa were put in tap water where some hatched within a few minutes and others continued to hatch over a period of two days.

The miracidium has two flame cells on each side of the body and in other respects is characteristic of this stage of species of the family SCHISTOSOMATIDAE. It is considerably larger than the miracidium of the human schistosomes, however, since it is 0.20 mm in length as compared with a length of about 0.13 mm for the miracidium of *Schistosoma haematobium*. The large size of the miracidia seems to be a characteristic of the avian schistosomes. The miracidia swim about actively and are attracted towards light.

On May 6, 1940, the following snails *Gyraulus parvus* (Say), *Menetus exacuous* (Say), and a species of *Physa* collected from Williams Creek near Madison, Wisconsin, were put in an aquarium containing water and plants from the habitat of the snails. The aquarium was aerated and kept at room temperature (about 70° F). Miracidia of the blackbird schistosome were introduced into this aquarium on May 6, 8, and 13, 1940. Snails in this experiment were first examined by autopsy on June 6, 1940, with the following results:

G. parvus
29 negative for cercariae
18 with monostome cercariae
1 with echinostome cercariae
2 with stylet cercariae
M. exacuous
3 negative for cercariae
1 with echinosome cercariae
1 with stylet cercariae
1 with monostome cercariae
1 with spirorchid cercariae
Physa sp.
1 large specimen negative for cercariae

The rest of the snails in this experiment were examined a week later on June 14, 1940, with the following results:

G. parvus
8 negative for cercariae
7 with monostome cercariae
M. exacuous
3 negative for cercariae
Physa sp.
1 large specimen negative for cercariae
4 small specimens negative for cercariae
2 small specimens with *C. gyrauli*

Controls for this experiment were collected on June 15, 1940, from precisely the same spot from which the experimental snails were col-

lected. One hundred and twenty-five *Physa* sp. of unquestionably the same generation as those used in the experiment were examined by removing the snail body from the shell and teasing it apart under a dissecting microscope. None of these snails was found to harbor *C. gyrauli* or any other cercaria that might be confused with it. Since two out of the six physas in the experiment and none of the 125 controls were infected with *C. gyrauli* it seems reasonably safe to assume that the cercariae in the experimental snails resulted from infections with the miracidia from the blackbird schistosome. These two infections were just reaching maturity which means that it required an interval of four to five weeks from the time the miracidia penetrated the snail host until cercariae were fully developed. This interval is in accordance with that for the development of *C. elvae* as shown by an earlier experiment (Brackett, 1940c).

The relationship of this schistosome to its snail intermediate host is confusing but very interesting. Since *C. gyrauli* was described from the snail *G. parvus* but was not found in physas from the same locality (Brackett, 1940b), it seems strange that in the above experiment this species should have developed in the physas and not in *G. parvus*. In view of Talbot's (1936) work in which he showed that three species of cercariae very similar in morphology developed in different snail hosts it might be suspected that we are dealing here with a species separate from but similar to *C. gyrauli* and one which requires a different snail host. Looking at Talbot's work further it is seen that in addition to having different hosts these three species (*C. elvae, C. stagnicolae,* and *C. physellae*), while almost indistinguishable morphologically, do differ quite strikingly in their behavior. Such is not the case with *C. gyrauli* and the cercariae developed in the above experiment for these latter cercariae agree perfectly in behavior with those which were used by the author (Brackett, 1940b) to establish the species *C. gyrauli.* A likely explanation is that a species of snail may not be equally susceptible to the invasion of miracidia of *P. gyrauli* at all stages in its developmental cycle. Consequently, it may be assumed that when *C. gyrauli* was found in 1939 in a large percentage of the snail *G. parvus* from Sun Prairie Pond, Wisconsin, the snails of this species were at a different stage in their cycle than those used in the experiment described in this paper. The physas examined from the Sun Prairie Pond in 1939 were not found to harbor *C. gyrauli* so, accordingly, they must have been at a stage in their cycle which was refractory to the miracidia of *G. gyrauli* when these miracidia were present.

There is another possibility that might be worthy of consideration. It was mentioned earlier that the miracidia of *G. gyrauli* are attracted towards light so that in their natural environment they would probably concentrate near the surface of the water. It seems possible that under

the natural conditions at the Sun Prairie Pond during the period these miracidia were in the water the physas might have been deeper in the water while the gyraulis were at the surface. An exact antithesis would then have to be assumed for the snails in the experimental aquarium. No observational records are available on this point.

It is obvious that no proved explanation for this host relationship is available. However, future work on the life cycles of the avian schistosomes may be expedited if this point is kept in mind and the conditions of the experiments broadened sufficiently in order to decrease the possibilities of failing to establish an infection even though the right hosts are being used. More extensive work on the life cycle of the blackbird schistosome would surely clarify the conditions necessary for successful establishment of its larval stages in the snail host.

If it is proved that the snail intermediate host is not equally susceptible to infection with schistosomes at all stages of its development, then the epidemiology of schistosome dermatitis becomes more complicated. It has been pointed out several times (Cort et al, 1940 and Brackett, 1941) that varieties of the snail *S. emarginata* are most important in outbreaks of swimmer's itch because they prefer sandy beaches and reach the height of their development during the swimming season. Also of significance is the fact that the dermatitis-producing cercaria *C. stagnicolae* in these snails reaches the peak of its development and emerges in greatest numbers during the height of the resorting season in Michigan and vicinity. It has been suggested (Cort et al, 1940) that the seasonal periodicity of this cercaria is due to the activity of the as yet unknown definitive host. For example, if the definitive host is a migratory bird it would be present on the beaches of northern Michigan only certain times of the year thus limiting the exposure of snails to an infection. If in addition to this we must consider the differential seasonal susceptibility of the snail intermediate host then the interrelationship between the factors influencing or favoring the completion of the life cycles of certain schistosomes is more complex and precise than had been suspected.

Distribution of *Gigantobilharzia gyrauli*

The adults of *G. gyrauli* were first found at Sun Prairie Pond, Wisconsin, in the spring of 1940. Earlier investigations showed three species of schistosome cercariae, *C. physellae, C. elongata,* and *C. gyrauli,* were to be found there but due to restricted collecting privileges only two species of adults were found. The author (Brackett, 1940c) gave evidence to suggest that *P. querquedulae,* which occurs commonly in the teal ducks from the Sun Prairie Pond, is the adult stage of *C. physellae* and concluded that the undescribed schistosome found in pied-billed grebes from this pond must be the adult stage of either *C. elongata* or *C. gyrauli.*

Since *C. gyrauli* has been shown to be the larva of *G. gyrauli* in blackbirds, then it seems possible that the grebe schistosome develops from *C. elongata.*

After finding that a large percentage of the blackbirds from the Sun Prairie Pond were infected with *G. gyrauli* (60 per cent of 7 red-winged blackbirds and 83 per cent of 6 yellow-headed blackbirds), these species from fourteen other localities were examined. This schistosome was found to be widely but unevenly distributed in central Wisconsin. Where it occurred it was found in a large percentage of the blackbirds but in other similar and often nearby localities it was completely absent (see Table 1).

TABLE 1.—*Blackbirds* examined for* P. gyrauli *in 1940*

Locality	Date	Number examined	Number positive	Per cent positive
	May			
Sun Prairie	6–8	7	4	69
	4–8	6 (yellow-headed)	5	83
Lake Monona	7–30	8	0	...
Dunn's marsh	7	4	0	...
Hammersley's marsh	16	5	0	...
Hope Lake	15–29	6	5	83
Red Cedar Lake	29	3	2	66
Sumner township Jefferson Co.	9	6	2 (light inf.)	33
Koshkonong station	9	3	0	...
Lake Poygan	13	3	2	66
Winneconne	12	2	2	100
Lake Butte des Morte	13	2	2	100
Rush Lake	11	5	1 (light inf.)	20
		5 (yellow-headed)	0	...
Horicon marsh	21	5	0	...
Hustiford Lake	22	2	0	...

* Unless otherwise indicated these are all red-winged blackbirds.

These irregularities in distribution do not seem to be due to chance but emphasize a fact which is already known to parasitologists and which was referred to earlier in this paper, namely, that the very special combination of factors permitting the completion of a trematode life cycle is not uniformly distributed. Those blackbirds examined early in the summer were probably carrying infections contracted the previous year because examinations of snails from the same areas during the spring up to the time the birds were examined showed that the cercaria of *G. gyrauli* was not yet escaping into the water. It is evident then that the blackbirds must have returned to the same place they had spent the previous year otherwise the distribution of the parasite would have been more uniform throughout the central Wisconsin marsh and lake region. The persistence with which many species of birds return to the same spot each year has been clearly shown by the banding records of the U. S. Biological Survey, but it seems of considerable interest and perhaps may prove to be

of some significance that such a definite relationship can be shown between a birds migratory habits and its parasitic infections.

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Explanation of Plates

All figures semidiagrammatic

Plate I

Fig. 1. *Pseudobilharziella waubesensis.* Anterior portion of female.
Fig. 2. *Pseudobilharziella waubesensis.* Anterior portion of male.
Fig. 3. *Pseudobilharziella kegonsensis.* Anterior portion of male.
Fig. 4. *Pseudobilharziella horiconensis.* a. Anterior portion of male. b. Posterior portion of male.
Fig. 5. *Pseudobilharziella burnetti.* Anterior portion of male.

Plate II

Fig. 6. *Gigantobilharzia gyrauli.* Anterior portion of female.
Fig. 7. *Gigantobilharzia gyrauli.* Anterior portion of male.
Fig. 8. *Gigantobilharzia lawayi.* Anterior portion of female.
Fig. 9. *Gigantobilharzia lawayi.* Anterior portion of male.

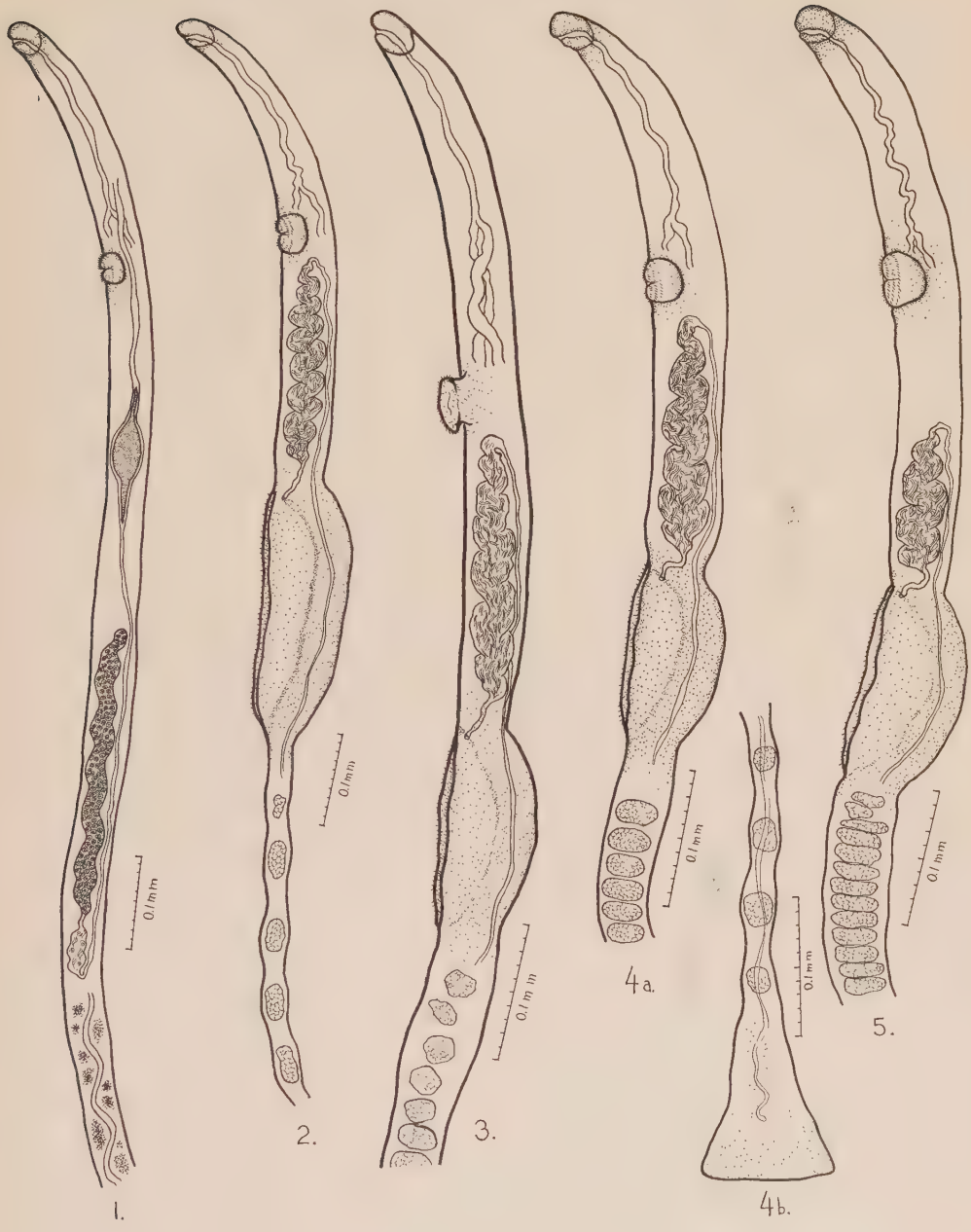

PLATE I

PLATE II

REACTIONS OF MAN TO THE FEEDING OF REDUVIID BUGS

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In reports of Kofoid and Whitaker (1936), Herms (1939), Wehrle (1939), and Wood (1941), observations have been recorded concerning the reactions of man after being bitten by various species of *Triatoma* (Hemiptera, Reduviidae). These reactions vary from a slight local redness at sight of bite to a severe illness lasting several days or weeks. In order to record the physical effects of the bite, the writer tested the feeding reactions of several species of uninfected bugs. The bug was allowed to feed on the left hand or arm while using the right hand to record observations.

OBSERVATIONS

On August 3, 1939, an adult female *Triatoma protracta* (Uhler) was placed upon a black-topped table beside my hand. The bug immediately extended its proboscis and began to probe the inside surface of the base of the little finger. A slight pricking sensation was felt as the tip of the proboscis was pressed against the skin and feeding started. After half a minute, the bug moved its body slightly and a more distinct pricking sensation was felt for 3 seconds. The antennae were held outward and slightly extended backwards while the body position was maintained by slight movements of the legs to adjust for slight movements of my hand. After 5 minutes, the bug's body could be seen to swell. After 6 minutes, the tips of the wings began rising above the noticeably rounded abdomen. Twelve and one-half minutes after contact, the bug withdrew its proboscis, having fed to capacity. Careful scrutiny of the finger at the exact point of contact did not reveal any evidence of a puncture, of swelling or redness. Repeated examinations failed to reveal any physical signs of a *Triatoma* having fed upon the finger.

Mr. "S" from Sanger, Fresno County, California, sent me one adult female *Triatoma protracta* with the following note, dated May 21, 1940: "This bug bit me on the hand last night. It evidently struck a vein for in ten minutes I was itching to my ankles and pains were shooting to my shoulder. This morning my hand is badly swollen. As yet the mark of the bite does not show but will when the swelling goes down. They affect my wife differently. She will not feel the bite usually but the next day will have a bad headache. She has learned to recognize the head-

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aches and when she has one we look and usually find the bug full of blood. I have known people in the neighborhood to become unconscious from the bites but thought it might be hysterics."

On July 8, 1939, an adult female *Triatoma protracta woodi* Usinger was placed beside my left hand which was palm down on the table. The bug extended its proboscis and immediately began probing. After trying several spots, it wandered away. No definite sensation could be felt as it moved the tip of its proboscis here and there over the finger. Turning my hand over, the bug was placed beside the little finger. The same probing technique was repeated, the bug seeming to avoid calloused areas of the finger. Soon its proboscis came to rest in a crease near the base of the little finger. Almost immediately, its body began to swell and after 5 minutes it withdrew its proboscis. At the site of puncture, there was a slightly reddened area 1 mm in diameter which soon disappeared so that a few minutes later no trace of the point of contact could be found. No pricking sensation of any kind was definitely felt at this feeding. On March 2, 1940, another adult female *T. p. woodi* fed on the inner surface of my left wrist. As the proboscis touched the skin and the bug pressed toward the wrist, a slight tickling sensation was felt for 1 minute. The bug began to feed immediately and no sensation of any kind could be felt indicating its presence. Eight minutes after contact a sensation was felt at the site of contact with the proboscis for a few seconds as if the skin had been touched lightly with a blunt, cold dissecting needle. Eleven and one-half minutes after contact the bug withdrew its proboscis, filled to capacity.

On September 10, 1939, an adult male *Triatoma longipes* Barber was placed on the table. The bug walked about for a few minutes then began slowly to wave its antennae back and forth as if sensing my warm hand. Very soon it extended its proboscis and touched the calloused undersurface of the thumb resting on the table. A slight but definite sensation of touch was felt as the proboscis contacted the thumb. Two and one-half minutes later a distinct tickling sensation was felt for 1 second (movement of the stylets in the epidermis?). Five minutes after contact a distinct tickling sensation was felt for 3 seconds. Six minutes after contact, the upper surface of the abdomen of the bug began to elevate, lifting the wing tips off the abdomen. At 6.5 minutes after contact, the bug withdrew its proboscis and walked away, being only about half full. No physical evidence of the contact could be seen.

On October 21, 1939, my left index finger was suspended in a jar containing 1 male and 1 female *Triatoma heidemanni* Neiva. Both bugs immediately reacted to the warmth by probing the tip of the finger. In 1 minute, both had made contact and started to feed. A definite sensation of something touching the tip of the finger was noticed, followed by a

slight tickling sensation for about 1 minute. One minute after contact, the abdomens of both bugs began to swell. Two and one-fourth minutes after contact, the wing tips of both were well raised above the ends of their abdomens. Three and one-half minutes after contact, a slight tickling sensation lasting several seconds was felt. Both bugs remained practically motionless during feeding. After 4¼ minutes, both bugs broke contact. No evidence of any physical damage to the finger at points of contact with the proboscides of the bugs could be found.

On May 18, 1940, an adult male *Triatoma gerstaeckeri* (Stål) made contact with the tip of my left index finger suspended in a glass jar. As it started to feed a sharp pricking sensation was noticed for about 1 minute (movement of stylets, ejection of saliva?). Thereafter, no sensitivity to the presence of the proboscis was noted. The only movements of the bug were slow waving of the antennae and movement of the legs to adjust body position for maintaining contact with slight movements of the finger. After 15½ minutes, the bug broke contact, having fed to capacity.

On July 13, 1940, an adult female *Triatoma sanguisuga* Lec. after 15 minutes of probing, retreating, testing and retesting the tip of the finger (as well as the surface of the nail) suspended in its jar, finally began to feed. Although there were many contacts of the tip of the bug's proboscis with the top of the finger, no sensation of any kind was felt until the proboscis stopped and the bug pressed the tip against the finger. This was followed by a slight tickling sensation for 3 seconds. The bug then remained perfectly motionless, the tip segment of its proboscis at right angles to the skin surface and the rest of the proboscis held at about a 45 degree angle. After 10 minutes, it slowly moved its antennae. Its abdomen was well rounded with the ends of the wings extending well above the top of the abdomen. After 23 minutes the bug seemed full but still held contact. After 29 minutes the finger was moved but the bug moved its body to maintain contact. At 30 minutes movements of the finger resulted in the bug pulling away. By this time, the abdomen of the bug was almost round in cross section like that of large nymphs after a full meal. On August 5, 1940, this same bug was placed on the black table top beside my forearm. Contact with my skin was made on the arm just below the elbow. There was no sensation of any kind as it began to feed. Five minutes later, the wing tips began to lift above the end of the abdomen. After 6 minutes, a tickling sensation was felt for 3 seconds. After 7 minutes, a slight "burning" sensation at the site of the bite was felt for 20 seconds. Eleven minutes after contact, a slight stinging sensation at the point of contact with the proboscis was felt for 15 seconds. After 12 minutes, the bug seemed filled to capacity but continued contact. Twenty-two minutes after contact, the external genitalia,

ordinarily not conspicuous, were forced to protrude by the pressure of the food on the abdominal organs. The conjunctivae between the abdominal segments were distended so that there were broad alternating light and dark bands across the posterior half of the abdomen. The bug pulled away from the arm of its own accord after 24 minutes of feeding. No physical reaction of any kind was found at the site of the bite and there was no feeling of discomfort during the feeding period or thereafter.

On July 23, 1940, an adult female *Triatoma rubida* (Uhler) began to feed almost as soon as my middle finger was suspended in its jar. The proboscis came to rest on the soft skin on top of the terminal joint. A slight pricking was felt for one-half minute, accompanied by a sensation of something touching the finger. The axis of the bug's body was at right angles to the axis of the finger. Its proboscis was held straight out except the tip, which tilted upward. Three and one-half minutes after contact the wing edges began to rise from the posterior edge of the abdomen. After 5 minutes, the abdomen was well rounded. The bug remained very quiet. After 10 minutes, it moved its hind legs as if to get a better stance. At 11½ minutes after contact, it pulled away. The point of contact was in a crease and showed a red spot of clotted blood 0.5 mm in diameter where the wound bled during or after contact with the bug's proboscis. For an area 1 mm in diameter around this spot the skin was slightly reddened. Rubbing the finger removed the clot but the small reddened zone, not swollen, remained for about 1½ hours. Another female *T. rubida* was placed on the table where it fed on the inside of my wrist. As it contacted the skin a definite pricking sensation was felt for 40 seconds followed by a slight tickling at the point of contact. The pricking was felt as the bug pushed its proboscis against the skin. When it stopped pushing, after contact was made, the pricking sensation stopped. When the arm moved, the bug adjusted its position by moving its body on its legs, rarely changing the position of its feet. Eight minutes after contact the bug pulled away, fed to capacity. There was a reddened zone 1 mm in diameter on the skin at the point of contact.

Five female *T. rubida* (2 infected with *Trypanosoma cruzi* Chagas) and one male *T. gerstaeckeri* were received from Mr. "R" who collected them from a house in Sanderson, Texas. Mr. "R" wrote me the following June 27, 1940: "The people living at this place are all in excellent health, and state that these suckers have sure dealt them misery."

On July 23, 1940, my left index and middle fingers were suspended in a jar with two female *Triatoma indictiva* Neiva. Both bugs scurried about the jar and tried to hide. After five minutes, one female moved up to a finger and then backed away without elevating her proboscis. Neither bug seemed inclined to feed. On July 26, the process was repeated for 11 minutes with no results. On August 1, attempts to feed

them in the daytime as above met with failure. This time the females did elevate their proboscides and probed the tip of a finger. They were then placed alongside the inner surface of the forearm and again one female probed several spots on the arm without making contact to feed. No sensation of any kind was felt as the bug moved its proboscis over the surface of the arm. Believing the insects might feed if it were night, I placed my index finger in their jar at 9:55 P.M. The jar was shaded from dim light so that the bugs were barely visible. Five minutes later, one female touched the tip of my finger with her proboscis and remained there for 20 seconds. The other female probed nearby, touching a finger for 30 seconds. Both broke contact and then tried to feed again at the edge of the nail. Two minutes later, as one female probed again, a slight tickling sensation was felt for 2 seconds but in a minute the bug pulled away, retreating into a fold of the paper toweling in the jar. Although a choice of fingers was offered, they did not feed. On August 3, similar results were experienced with the same two female *T. indictiva.* On August 7, noting that the bugs seemed weak, they were placed with a white mouse but neither one fed.

On August 1, 1940, one adult male *Paratriatoma hirsuta* Barber was placed on a pleated piece of paper toweling near the inside of my left wrist. The bug immediately extended its proboscis, inserting it on the undersurface of the wrist. A sharp pricking sensation was felt for 4 seconds, followed by a slight tickling sensation for 5 seconds. One and one-half minutes after contact, the abdomen of the bug began to swell, elevating the wings off the abdomen. By three minutes, the abdomen was markedly distended so that the upper sides showed the red color of the blood in the internal organs beneath. Six minutes after contact, there was a sharp pricking sensation for about 2 seconds. The bug stopped feeding in $6\frac{1}{4}$ minutes, filled to capacity. Another adult male *P. hirsuta* began feeding almost immediately. With the first contact on the skin, there was a brief sharp pricking sensation lasting 10 seconds. The bug immediately began to fill up. Two minutes after contact, the abdomen could be seen enlarging, causing the ends of the wings to be raised. Five minutes after contact, a sensation of something touching lightly at the point of contact was felt for 5 seconds. This bug fed to capacity in $9\frac{1}{2}$ minutes and pulled away. In both cases, no physical disturbance to my skin of any kind could be found at the time of feeding. Forty hours after the bug stopped feeding, a severe itching of the underside of the left wrist directed my attention to the two sites where *Paratriatoma* had fed. The area around each bite was swollen and inflamed. A slight, continuous itching sensation of these areas was felt from forty hours on. Fifty-two hours after the bug had stopped feeding, the round, swollen, reddened areas measured 4 mm in diameter and the itching was less intense. At 70

hours, the "bumps" measured 3 mm in diameter, were still firm but only slightly inflamed. At 94 hours, at one site a hard lump, 3 mm in diameter, was evident. At the other site, there was only a slight elevation and the area affected measured 2 mm in diameter. At 118 hours, both elevated areas measured 2 mm across and appeared bluish red in contrast to the former pinkish red. The swelling at one site had disappeared but the other site was still firm and hard although somewhat smaller than at the previous observation. Seven days after being bitten, there were two purplish-red areas about 2 mm in diameter with only the central point raised but no physical sign of an actual puncture mark could be found. At 15 days, two discolored areas at the site of the bites were distinctly visible. They measured 2 mm in diameter and were gradually fading, especially at the edges. At 17 days, the one bite which had remained swollen and hard longer than the other was now barely visible. The other site was a deeper red in color and showed a hard, swollen, centrally elevated region 1 mm in diameter after having shown no swelling for several days. At 19 days, there was no trace of the one bite. The other bite showed a swollen, reddened, elevated area 2 mm in diameter which was recognizable only as a tiny pink dot at 30 days.

DISCUSSION

Since it has been shown that these bugs are vectors of Chagas' disease (F. D. Wood, 1934; Kofoid and Whitaker, 1936; Packchanian, 1939; S. F. Wood, 1941) in the United States, distinction should be made between symptoms which are purely reactions to physical and chemical (salivary secretions) contacts with the bugs and possible reactions to disease organisms that they may carry. The method of transmission of *Trypanosoma cruzi,* causative agent of Chagas' disease, is by contamination of the bug bite, of a skin abrasion or of a mucous membrane by infective feces of the bug. As has been emphasized recently by Mazza (1939), a very prevalent and easily recognizable symptom of Chagas' disease is the swelling of one or both eyelids (unilateral or bilateral palpebral edema) after contact with infected insects.

SUMMARY

The writer experienced no marked physical discomfort *during* feedings of *Triatoma protracta, T. p. woodi, T. longipes, T. heidemanni, T. gerstaeckeri, T. sanguisuga, T. rubida,* and *Paratriatoma hirsuta* at different times upon various areas of his left fingers, hand and forearm. In every case but one, there was no marked injury due to the bite. In the case of *Paratriatoma hirsuta* Barber, there was a local irritation of the skin at the site of the bites, but there was no general reaction of any kind.

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STRIGEID TREMATODES OF THE ALLIGATOR, WITH REMARKS ON THE PROSTATE GLAND AND TERMINAL PORTIONS OF THE GENITAL DUCTS

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Within recent years at least five species of strigeid trematodes have been made available for study by the writers. These worms were recovered from the small intestine of the alligator, *Alligator mississipiensis* (Daudin), from three localities, Silver Springs, Florida, Tallahassee, Florida, and Midville, Georgia. Since all five species of the flukes possess a prostate gland, they have been placed in the family of PROTERODIPLOSTOMIDAE Dubois, 1936. Three of the species are considered new to science and two of these are placed in a new genus. A description of the new genus and species follows.

Family PROTERODIPLOSTOMIDAE Dubois, 1936
Subfamily PROTERODIPLOSTOMINAE Dubois, 1936
Pseudoneodiplostomum acetabulata n. sp.
(Figs. 1–2)

Specific diagnosis: Pseudoneodiplostomum. Elongated strigeid fluke with body divided into two segments, anterior segment of which is relatively thin and flat; posterior segment almost cylindrical. Total length of body 5.50 ± .01 (4.80–5.96)* mm. Anterior segment broadest in region of holdfast organ, tapering anteriorly to a much narrower and more rounded anterior margin, 2.78 ± .005 (2.44–2.96) mm long by 1.02 ± .004 (0.83–1.22) mm in greatest width. Posterior segment slightly overlapped by anterior segment, attached to anterior segment by anterio-ventral margin, widest anteriorly, with almost parallel sides but tapering slightly toward posterior end, 2.74 ± .006 (2.24–3.07) mm long by 534 ± .09 (320–770) μ in greatest width. Oral sucker muscular, from 130 to 160 μ in diameter, at anterior margin of body. Prepharynx short or absent. Pharynx 87 to 110 μ in diameter. Esophagus short, about same length as pharynx. Ceca slender tubes, curving around acetabulum and holdfast organ, reaching to about level of middle of prostate in hindbody. Acetabulum large and muscular, 410 ± .01 (370–450) μ in diameter, situated 0.96 ± .003 (0.77–1.06) mm behind anterior margin of body. Holdfast organ broadly elliptical, muscular, with about 12 papillae bordering central cavity, 680 ± .002 (570–840) μ long by 610 ± .002 (500–740) μ wide, located 470 ± .004 (410–510) μ behind acetabulum, just in front of hindbody. Proteolytic gland large, multilobed, dorsal to posterior margin of holdfast organ.

Ovary dorsal in position, to left of midline, 127 ± .005 (87–168) μ long by 173 ± .02 (110–306) μ wide, placed 0.62 ± .006 (0.27–1.13) mm behind union of two body segments. Oviduct long, giving rise to Laurer's canal about midway between ovary and oötype, forming oötype in intertesticular space. Shell gland and yolk reservoir rather large, between the testes. Uterus forming several transverse loops in as-

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* Measurements are presented in two ways. 1. The mean with its probable error. 2. The range of measurements (from smallest to largest) in parentheses. In computing the probable error the number of degrees of freedom is taken into account.

cending to anterior limits of hindbody, then forming more transverse loops in descending to genital pore, surrounded by muscle in area of prostate to form metraterm. Metraterm about as long as prostate. Ova 87 to 97 μ long by 44 to 61 μ wide, several dozen usually present in uterus. Vitellaria follicular, distributed into both body segments, extending as far forward as caudal boundary of acetabulum in anterior segment and posteriorly to level of prostate in hindbody; vitellaria in posterior segment in two main bands, dorsal, lateral and ventral to ceca behind ovary but may become continuous band across dorsum of body in anterior part of hindbody.

Testes in tandem, close behind ovary, triangular to rectangular (seldom round) in shape, in middle of hindbody. Anterior testis 220 ± .01 (170–330) μ long by 350 ± .003 (240–500) μ wide. Posterior testis 213 ± .02 (190–339) μ long by 338 ± .01 (200–504) μ wide, situated 180 ± .02 (84–300) μ behind anterior testis. Vasa efferentia uniting in dorsal area of intertesticular space to form small seminal reservoir. Vas deferens arising from reservoir, passing posteriorly almost parallel to uterus in ventral aspect of body, gradually increasing in width, becoming dilated to form seminal vesicle in area immediately behind testes. Seminal vesicle considerably coiled, constricting into ejaculatory duct near anterior limit of prostate. Ejaculatory duct with thin, muscular wall in central part of its length, joining prostatic canal at level approximating one-fourth length of prostate from caudal end. Prostate rather large, 310 to 450 μ long by 120 to 160 μ wide, with heavy muscular wall and medium-large central cavity. Prostatic canal joined by uterus at end of prostate, forming hermaphroditic canal. Hermaphroditic canal opening at summit of genital papilla into rather large atrium. Genital atrium dorsal in position. Prostate gland, ejaculatory duct (muscular portion) and metraterm surrounded by their own individual masses of gland cells; prostate surrounded by more of these cells than is the case with other two structures.

Host: Alligator mississipiensis (Daudin).
Habitat: Small intestine.
Locality: Silver Springs, Florida.
Additional specimens: Tallahassee, Florida, and Midville, Georgia.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44968. *Paratype:* 44969.

The present species is considered distinct from the other two species, *Pseudoneodiplostomum siamense* (Poirier) and *P. thomasi* (Dollfus), now assigned to the genus, due to its larger body size, the more posterior distribution of the vitellaria in the hindbody and the much larger acetabulum. More specifically, *P. acetabulata* can be separated from *P. thomasi* by its larger body size, the more elongated hindbody, the more posterior position of the gonads, the larger ventral sucker and the greater posterior extension of the vitellaria in the hindbody. From *P. siamense* the present species differs in regards to the larger body size, the much larger ventral sucker, the more posterior distribution of the vitellaria, the absence of large, flaring, finger-like papillae about the margin of the holdfast and in the nature of the prostate.

Subfamily Polycotylinae Monticelli, 1888
Polycotyle ornata Willemoes-Suhm, 1870
(Figs. 3–4)

Specific diagnosis: Polycotyle. Small to medium-sized, bisegmented strigeid fluke with slight dorsal curvature of posterior segment, 2.30 ± .01 (1.81–3.12) mm in length. Anterior segment 1.13 ± .006 (0.87–1.48) mm long by 550 ± .003 (430–650) μ wide in region of holdfast organ. Posterior segment 1.19 ± .008 (0.96–1.60)

mm in length, attached to posterio-dorsal margin of anterior segment, partly overlapped by forebody. Oral sucker at anterior margin of body, approximately 35 μ in diameter. Prepharynx short or absent. Pharynx same size or slightly smaller than oral sucker. Esophagus about three times as long as pharynx. Ceca slender tubes, curving around holdfast organ, reaching to near level of genital papilla. Ventral sucker transversely oval, 75 ± .004 (52–87) μ long by 103 ± .006 (87–130) μ wide, situated 484 ± .02 (390–610) μ behind anterior margin of body. Holdfast organ elongated elliptical, muscular, with about 12 to 15 short papillae bordering central cavity, 243 ± .02 (190–350) μ long by 183 ± .01 (174–260) μ wide, situated 90 ± .01 (61–174) μ behind acetabulum and 169 ± .01 (130–240) μ in advance of caudal margin of forebody. Proteolytic gland conspicuous, median in position, dorsal to caudal margin of holdfast organ. Nerve ring and lateral nerve trunks visible in anterior part of forebody.

Ovary transversely oval, 98 ± .006 (87–130) μ long by 126 ± .006 (100–150) μ wide, located slightly to right of midline at caudal margin of forebody, from 43 to 240 μ in front of testes. Shell gland immediately behind ovary and slightly more to right in position. Yolk reservoir large, to left of position of shell gland, immediately behind ovary. Oviduct short, giving rise to Laurer's canal at about level of caudal boundary of ovary, forming oötype in space between ovary and testes. Uterus slightly convoluted, ascending to extreme anterior margin of hindbody before descending to genital pore, with small sphincter muscle immediately in front of its union with the ejaculatory duct, containing up to 10 ova. Ova 87 to 96 μ in length. Vitellaria follicular, confined to anterior segment, from level of acetabulum to caudal boundary of forebody, more concentrated in region of holdfast organ.

Testes posterior to shell gland and yolk reservoir, in tandem or diagonal positions; anterior testis displaced slightly to right of midline and posterior one a little to left of midline, often with levels overlapping somewhat but not infrequently with posterior testis as much as 50 μ behind anterior testis. Anterior testis transversely ovoid to triangular, 154 ± .01 (113–240) μ long by 205 ± .005 (174–220) μ wide. Posterior testis of same shape as anterior, 146 ± .01 (113–191) μ long by 201 ± .005 (174–230) μ wide, situated 776 ± .004 (620–940) μ in advance of caudal margin of hindbody. Vasa efferentia arising from main body of their respective testis, ascending to space between ovary and testes to form small seminal reservoir. Vas deferens descending from reservoir to space behind caudal testis, forming seminal vesicle. Seminal vesicle greatly convoluted, narrowing into ejaculatory duct which proceeds posteriorly alongside of prostate to discharge into uterus. Canal formed by fusion of uterus and ejaculatory duct short, discharging almost immediately into canal of prostate. Prostate muscular, elongated, approximately 450 μ long by 52 μ in greatest width, surrounded by rather large, evacuolated prostatic gland cells. Prostatic canal with corrugated, cuticular lining, joining with canal of uterus and ejaculatory duct to form hermaphroditic canal. Hermaphroditic canal short, opening to outside at summit of the genital papilla into rather large, dorsal atrium. Posterior body segment with median, dorsal row of 17 to 19 suckers, beginning at level of ovary and terminating at atrium. Largest sucker in posterior position of row, 130 μ in diameter; suckers diminish in size toward anterior end of row until smallest measures only 20 μ in diameter. In sectioned material two small suckers may be observed between last two suckers in dorsal row. A single, large sucker, measuring 130 by 170 μ, is placed in the atrium, immediately behind genital papilla.

Host: Alligator mississipiensis (Daudin).
Habitat: Small intestine.
Locality: Silver Springs, Florida.
Additional material: Tallahassee, Florida, and Midville, Georgia.

The material collected by us is considered to be identical with that described by Willemoes-Suhm (1870), Poirier (1886), Dubois (1936) and others, although none of the specimens in the present collection are

as large as those considered by these writers. In practically every detail our material is smaller. Only one other significant difference is to be noted; there are at least seventeen suckers in the median, dorsal row on the posterior segment of the body, whereas only fifteen suckers have been reported previously as constituting this row. In some of our specimens we have been able to count as many as twenty of these suckers in living material, although on fixation and mounting it is found that the density of the stain in the ovary and its complex renders the more anterior suckers more difficult to see. In one of the sectioned worms eighteen suckers are present while in another there are nineteen. In the sectioned specimens two very small suckers are demonstrated in the area between the last two suckers in the dorsal row.

Crocodilicola pseudostoma (Willemoes-Suhm, 1870)

(Figs. 5–6)

Specific diagnosis: Crocodilicola. Small strigeid fluke with unsegmented body or with body segmentation only feebly indicated in region of ovary where thin, flat anterior segment unites with almost cone-shaped posterior segment, measuring 1.60 to 1.80 mm in length for entire body. Anterior segment rounded to slightly pointed anteriorly, broadening posteriorly to attain maximum width in region of holdfast organ, 1.00 to 1.20 mm long by 560 to 620 μ in width at widest part. Posterior body 710 to 740 μ long by 400 to 460 μ wide, widest in anterior region, tapering to blunt point at posterior end. Oral sucker 43 μ in diameter, located at extreme anterior margin of forebody. Prepharynx short or absent. Pharynx 35 μ in diameter. Esophagus about 70 μ long. Ceca slender tubes, reaching to near genital papilla. Acetabulum transversely oval, 60 to 70 μ long by 113 to 130 μ wide, located 450 to 590 μ behind anterior margin of body. Holdfast organ slightly ovoid, about 200 μ in diameter, situated 190 to 220 μ behind acetabulum and very near caudal boundary of forebody. Nerve ring and lateral nerve trunks visible in forebody to vitellaria.

Ovary transversely oval, 70 μ long by 140 to 160 μ wide, in midline close behind caudal limit of vitellaria. Oviduct long, extending posteriorly, dorsal to anterior testis, to intertesticular space, giving rise to Laurer's canal midway between ovary and oötype. Oötype, shell gland and yolk reservoir between testes. Uterus passing out to right and up to anterior margin of hindbody, swings to left across body to ventral position and downward to genital pore, developing sphincter muscle just before discharging into hermaphroditic canal at end of prostate, containing relatively few ova. Ova 96 to 104 μ long. Vitellaria follicular, confined to forebody, from level of acetabulum to caudal margin of forebody, more concentrated about ceca and holdfast organ. Proteolytic gland conspicuous, at caudal boundary of holdfast organ.

Testes in tandem, transversely oval; anterior testis 130 to 160 μ long by 360 to 370 μ wide, located 30 to 50 μ behind ovary; posterior testis 130 to 174 μ long by 330 to 350 μ wide, separated from anterior testis by shell gland and yolk reservoir, placed 240 to 280 μ in advance of caudal boundary of hindbody. Vasa efferentia arising from opposite sides of their respective testis, passing forward to form small seminal reservoir in space between ovary and testes. Vas deferens arising from seminal reservoir, passing downward in ventral aspect of body, forming greatly dilated seminal vesicle immediately behind caudal testis. Ejaculatory duct arising as narrow tube from seminal vesicle, passing posteriorly between uterus and prostate, joining prostatic canal at about one-third the length of that canal from its posterior end, developing slightly muscular wall just before joining pro-

static canal. Prostate ellipsoidal pouch with comparatively thick, muscular wall and large central cavity. Central cavity with corrugated, cuticular lining. Prostatic gland cells numerous, small, greatly compact in certain areas about prostate. The fused prostatic canal and ejaculatory duct joins uterus at end of prostate to form hermaphroditic canal. This canal is short and opens at summit of short genital papilla into small genital atrium.

Host: Alligator mississipiensis (Daudin).

Habitat: Small intestine.

Locality: Silver Springs, Florida.

Although only four mature specimens of this species were recovered from a single alligator, there can be but little doubt that we are dealing with *Crocodilicola pseudostoma.* Two of our mature specimens were used for sectioning and the other two were mounted *in toto.* Our specimens are somewhat smaller in total body size than reported in the literature, but being unable to tell whether or not our specimens are fully matured (of about a dozen specimens collected all but four are considered as immature forms), we prefer to describe them as belonging to the species to which they are assigned. In so far as we can determine our specimens depart from the typical form as described by Dubois (1936b) only in regards to the more transversely oval testes and in the fusion of the ejaculatory duct with the prostatic canal instead of with the uterus.

Pseudocrocodilicola n. g.

Generic diagnosis: POLYCOTYLINAE. Small, bisegmented strigeid flukes with lanceolate anterior segment which is longer than the elongated cylindrical posterior segment. Acetabulum in middle or in advance of middle of anterior segment. Holdfast organ in anterior limit of last third of anterior segment, with 10 to 12 papillae bordering central cavity. Proteolytic gland present. Vitellaria follicular, confined to anterior segment. Gonads in tandem, in posterior segment; ovary in advance of testes, separated from testes by small seminal reservoir. Shell gland and yolk reservoir between testes. Laurer's canal present. Uterus ascending to anterior limit of posterior segment, descending to genital pore, containing relatively few ova. Seminal vesicle large, between testes and prostate. Ejaculatory duct discharging into prostatic canal. Prostate small, muscular, ellipsoidal in shape, surrounded by relatively few but large gland cells. Uterus and prostatic canal unite to form hermaphroditic canal. Hermaphroditic canal surrounded by a secondary pouch of muscle or a parenchyma of spongy-like tissue containing muscle fibers, dilated into genital cloaca, discharging into small, almost negligible atrium.

Type species: Pseudocrocodilicola americaniense n. sp.

Additional species: Pseudocrocodilicola georgiana n. sp.

Pseudocrocodilicola americaniense n. sp.

(Figs. 7–8)

Specific diagnosis: Pseudocrocodilicola. Small, lancet-shaped strigeid fluke with bisegmented body, 3.14 ± .03 (2.88–3.60) mm in total length. Anterior segment very thin and flat anteriorly, almost parallel sides, slightly overlapping posterior segment ventrally, 2.30 ± .02 (2.17–2.44) mm long by 480 ± .01 (430–520) μ wide. Posterior segment cylindrical, attached to posterio-dorsal surface of forebody, 0.95 ± .005 (0.83–1.24) mm long by 250 ± .01 (220–270) μ wide. Oral sucker 44 to 52 μ in diameter. Prepharynx short or absent. Pharynx 44 to 61 μ in diameter. Esophagus 130 to 174 μ long. Ceca slender tubes, reaching to level of prostate in

posterior segment. Acetabulum weakly muscular, 100 μ in diameter, located 1.00 ± .004 (0.80–1.20) mm behind anterior margin of forebody. Holdfast organ elliptical, muscular, with about 12 papillae bordering central cavity, 340 ± .007 (310–370) μ long by 210 ± .003 (190–220) μ wide, located 480 ± .003 (310–700) μ behind ventral sucker and 340 ± .01 (290–400) μ in advance of caudal margin of forebody. Proteolytic gland small but well developed, dorsal to caudal margin of holdfast organ. Nerve ring and lateral nerve trunks visible in forebody to level of vitellaria.

Ovary 87 to 96 μ in diameter, to left of midline in anterior part of hindbody. Oviduct descending dorsally to anterior testis, giving rise to oötype in intertesticular space. Laurer's canal present. Shell gland and yolk reservoir between testes. Uterus ascending to anterior extremity of hindbody before descending in ventral aspect of hindbody to genital pore, containing up to 16 ova. Ova 97 to 104 μ long by 52 to 70 μ wide. Vitellaria follicular, in forebody from caudal boundary of forebody to or just behind acetabulum.

Testes small, transversely oval, one behind other in anterior two-thirds of hindbody. Anterior testis 180 ± .004 (160–220) μ long by 220 ± .008 (190–224) μ wide, separated from ovary by small seminal reservoir. Posterior testis 190 ± .01 (180–220) μ long by 210 ± .006 (170–230) μ wide, from 50 to 100 μ behind anterior testis. Vasa efferentia arising from opposite sides of their respective testis, passing forward to form small seminal reservoir in space immediately behind ovary. Vas deferens descending from reservoir, gradually increasing in width in descending to seminal vesicle. Seminal vesicle S-shaped, in space behind caudal testis, constricting into ejaculatory duct. Ejaculatory duct passing from left to right side of body, then turning rather sharply inward to join prostatic canal just caudal to middle of prostate. Prostate small, muscular, ellipsoidal pouch, 200 to 300 μ long by 100 to 150 μ in greatest width, surrounded by numerous rather large and scattered gland cells. Atrium small. Genital papilla slight elevation into atrium. Genital pore at summit of papilla, passing inward as hermaphroditic canal. Hermaphroditic canal surrounded by muscular pouch that continues along uterus for short distance, becoming dilated at point of union of uterus with prostatic canal. Musculature of prostate penetrates secondary pouch to form a prostatic papilla in dilated part of hermaphroditic canal.

Host: Alligator mississipiensis (Daudin).

Habitat: Small intestine.

Locality: Midville, Georgia.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44970. *Paratype:* 44971.

Pseudocrocodilicola georgiana n. sp.

(Figs. 9–10)

Specific diagnosis: Pseudocrocodilicola. Small lancet-shaped, bisegmented strigeid fluke, 1.58 ± .004 (1.44–1.77) mm in total length. Anterior segment thin and flat anteriorly, widest in region of holdfast, slightly overlapping anterior part of hindbody, 1.08 ± .004 (0.96–1.22) mm long by 300 ± .003 (280–310) μ in greatest width. Posterior segment cylindrical, attached to posterio-dorsal surface of anterior segment, 610 ± .003 (460–810) μ long by 160 ± .01 (160–170) μ wide. Oral sucker 35 to 43 μ in diameter. Prepharynx short or absent. Pharynx same size as oral sucker. Esophagus 70 to 130 μ long. Ceca narrow tubes, extending to middle of prostate in posterior segment. Acetabulum weakly muscular, 61 μ in diameter, 520 ± .002 (440–570) μ behind anterior margin of forebody. Holdfast organ elliptical, muscular, with about 12 papillae bordering central cavity, 146 ± .03 (130–160) μ long by 96 to 100 μ wide, located 162 ± .005 (130–180) μ behind acetabulum and 188 ± .04 (170–260) μ in advance of caudal margin of forebody. Proteolytic gland small, dorsal to caudal margin of holdfast organ.

Ovary in anterior part of hindbody, slightly to right of midline, 70 ± .02 (61–80) μ in diameter. Oviduct arising from middle of ovary, descending dorsally to anterior testis, giving rise to oötype in space between testes. Laurer's canal

present. Shell gland and yolk reservoir intertesticular. Uterus ascending to anterior limit of hindbody, descending in ventral aspect of body to genital pore, containing up to 11 ova. Ova 70 to 96 μ in length. Vitellaria follicular, confined to forebody, from caudal margin of forebody to level 87 to 174 μ in advance of acetabulum.

Testes in tandem, in anterior three-fifths of hindbody; anterior testis 125 ± .004 (113–140) μ long by 138 ± .02 (130–150) μ wide, abutting ovary or up to 60 μ behind that organ; posterior testis separated from anterior testis by shell gland and yolk reservoir, 142 ± .01 (130–150) μ long by 147 ± .003 (130–165) μ wide, located 224 ± .01 (174–320) μ from caudal boundary of hindbody. Vasa efferentia uniting in area between ovary and testes, forming small seminal reservoir from which vas deferens arises. Vas deferens descending alongside uterus to area immediately behind testes, forming seminal vesicle. Ejaculatory duct emerging from seminal vesicle to join prostatic canal at anterior end of prostate, entering prostate from anterio-ventral aspect. Prostate muscular, ellipsoidal in shape, about 100 μ long, surrounded by relatively few, scattered gland cells. Genital atrium small, with only slight indication of genital papilla. Hermaphroditic canal rather long, greatly dilated, surrounded by parenchyma of spongy-like tissue containing muscle fibers (the secondary pouch), passing inward through secondary pouch from summit of papilla to end of prostate, receiving both the uterus and prostatic canal at that point. Musculature of prostate penetrates secondary pouch.

Host: Alligator mississipiensis (Daudin).
Habitat: Small intestine.
Locality: Midville, Georgia.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 44972. *Paratype:* 44973.

Pseudocrocodilicola georgiana is very similar to *P. americaniense* but differs from that species in regards to several important details. It is considerably smaller of body size than *P. americaniense.* At first this difference was not considered as important since a goodly number of young adults and immature specimens of the larger species was found in which the body size was identical with that of the more mature specimens of *P. georgiana.* Regardless of body size the relative position of the acetabulum and vitellaria remained constant in each species. In *P. americaniense* the vitellaria extends only to the acetabulum while in *P. georgiana* these glands may extend as far as midway between the acetabulum and the anterior margin of the forebody; they never terminate at or behind the acetabulum. It is true that in the larger species the acetabulum may be as much as three times as far away from the holdfast organ as is the case in *P. georgiana,* and this may account for the difference noted in the forward distribution of the vitellaria in the smaller species. However, here again is a constant difference in the specimens of the two species regardless of body size. The only other noteworthy difference between the two species has to do with the terminal parts of the genital ducts. In *P. georgiana* the ejaculatory duct fuses with the prostatic canal at its anterior end; the duct penetrates the prostate at its anterio-ventral margin. On the other hand, the ejaculatory duct in *P. americaniense* penetrates the prostate and fuses with the prostatic canal posterior to the middle of the prostate.

The genus *Pseudocrocodilicola* is considered to be more closely related to the genus *Crocodilicola* than to the other genera of the subfamily Poly-

COTYLINAE because of the general appearance of the body and its internal organization. However, it is considered to be distinct from that genus because of the more distinct bisegmentation of the body, the more forward position of the holdfast organ, the more cylindrical posterior body segment and the nature of the prostate and associated ducts. Dubois (1936b) described the ejaculatory duct in *Crocodilicola* as entering the uterus rather than the prostatic canal. The duct so formed by these two organs fused with the prostatic canal before they opened into a rather large genital atrium. In our material, identified as *C. pseudostoma,* however, this is not the case, for certainly the ejaculatory duct fuses with the prostatic canal and then this canal is joined by the uterus to form the hermaphroditic canal. Accordingly, no structure comparable with the secondary pouch has been observed in *Crocodilicola.* Such a pouch has developed to very conspicuous proportions in *Pseudocrocodilicola.* In regards to the secondary pouch, we have been unable to find reference to any structure that may be interpreted as being a counterpart of this structure. A cirrus pouch has been described for the species *Heterodiplostomum lanceolatum* by Dubois (1936b), and this bears some resemblance to the secondary pouch of the present genus. In this species, however, the ejaculatory duct does not unite with the prostatic canal as in *Pseudocrocodilicola,* but fuses with the uterus. The hermaphroditic canal so formed discharges into the atrium at the base of the genital papilla while the prostatic canal ramifies the cirrus pouch before opening into the atrium at the summit of the papilla. These two genera further differ in that the vitellaria are distributed into both segments of the body in *Heterodiplostomum.*

DISCUSSION

For the strigeid flukes parasitic in the Reptilia, Dubois (1936a) proposed the family PROTERODIPLOSTOMIDAE. Due primarily to differences noted in the size and shape of the holdfast organ (tribocytic organ), the presence or absence of papillae bordering the cavity of the holdfast organ and differences in the distribution of the vitellaria, Dubois recognized two supersubfamilies and three subfamilies in the family group. In the first supersubfamily, PROTERODIPLOSTOMIDI, only those species were included in which the holdfast organ was small to medium-sized and which bore papillae on the margin of its cavity. The supersubfamily was divided into the subfamilies PROTERODIPLOSTOMINAE Dubois, 1936, in which the vitellaria were distributed to both segments of the body, and POLYCOTYLINAE Monticelli, 1888, in which the vitellaria were confined to the anterior segment of the body. The subfamily OPHIODIPLOSTOMINAE Dubois, 1936, in which the holdfast organ is massive and often greatly elongated, and is devoid of papillae, constituted the only subfamily in the supersubfamily OPHIODIPLOSTOMIDI.

The same year Dubois (1936b) diagnosed the family PROTERODIPLOSTOMIDAE to include those strigeid flukes parasitic in reptiles in which the anterior body segment was flat and foliiform, the testes were spherical or ovoid and a prostate, located dorsally to the ejaculatory duct, was present. In the family proper, as now outlined, nine genera were included as follows: 1. *Proterodiplostomum* Dubois, 1936, 2. *Mesodiplostomum* Dubois, 1936, 3. *Prolecithodiplostomum* Dubois, 1936, 4. *Cystodiplostomum* Dubois, 1936, 5. *Herpetodiplostomum* Dubois, 1936, 6. *Paradiplostomum* La Rue, 1926, 7. *Ophiodiplostomum* Dubois, 1936, 8. *Petalodiplostomum* Dubois, 1936, and 9. *Heterodiplostomum* Dubois, 1936. To these nine genera were assigned twelve species, of which all but three were considered as new. In the "addenda" of the family was placed the genera *Polycotyle* Willemoes-Suhm, 1870, *Crocodilicola* Poche, 1925, *Proalarioides* Yamaguti, 1933, and *Pseudoneodiplostomum* Dubois, 1936. These four genera contained five species. In addition to these genera and species, six inadequately described species were placed in the "species inquirendae."

Later the same author (1938b) diagnosed the family PROTERODIPLOSTOMIDAE to include those diplostome parasites of reptiles that are characterized by the presence of a paraprostate, located dorsally to the ejaculatory canal, the efferent canal of which constitutes a third genital canal (exceptions: *Mesodiplostomum gladiolum* Dubois, 1936, and *Proalarioides serpentis* Yamaguti, 1933), and having testes that are spherical or ovoid but never lobed. In the three already mentioned subfamilies the following genera were assigned: 1. *Proterodiplostomum,* 2. *Mesodiplostomum* and 3. *Pseudoneodiplostomum* to the subfamily PROTERODIPLOSTOMINAE; 1. *Polycotyle,* 2. *Crocodilicola,* 3. *Proalarioides,* 4. *Cystodiplostomum,* 5. *Herpetodiplostomum,* 6. *Paradiplostomum* and 7. *Prolecithodiplostomum* to the subfamily POLYCOTYLINAE; and 1. *Ophiodiplostomum,* 2. *Heterodiplostomum* and 3. *Petalodiplostomum* to the subfamily OPHIODIPLOSTOMINAE. The species inquirendae remained about as previously outlined.

To this fairly homogeneous group of strigeid flukes from the Reptilia the present paper adds *Pseudoneodiplostomum acetabulata* n. sp., assigned to the subfamily PROTERODIPLOSTOMINAE, and *Pseudocrocodilicola americaniense* n. g., n. sp. and *P. georgiana* n. sp., assigned to the subfamily POLYCOTYLINAE.

Any real attempt to evaluate the more than usual elaborate system of classification as proposed by Dubois (1936a, 1936b, 1938b) for the strigeid trematodes does not seem necessary at this time. We are more particularly concerned with those forms reported from the REPTILIA, and since all of our material came from a single host species, *Alligator mississipiensis,* we do not feel justified in expressing an opinion concerning the

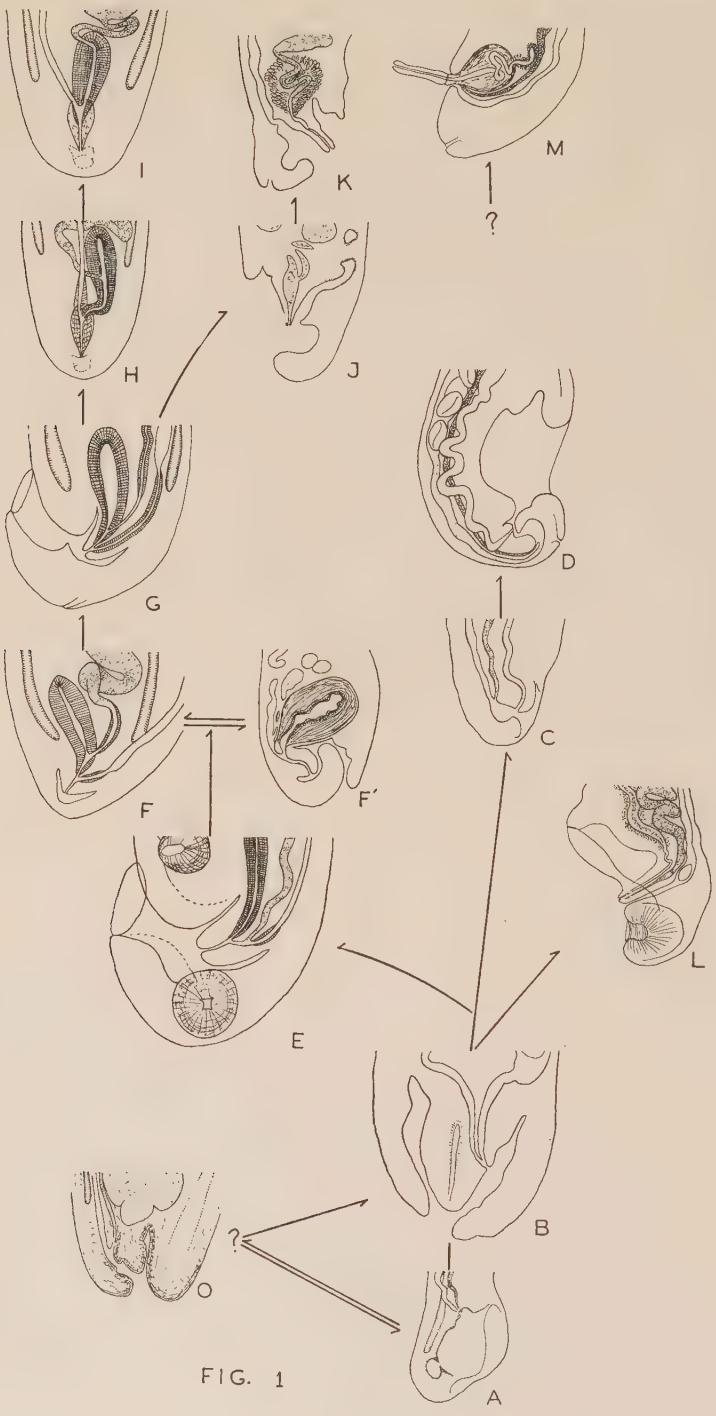

FIG. 1

multitude of genera proposed for the relatively small number of species from these hosts. It does seem logical, however, to place this rather homogeneous assemblage of parasites in a single family as Dubois already has done. With but two possible exceptions among the more adequately described species all members of the family group possess a common character in the presence of the prostate gland, a structure not to be found elsewhere among the multitude of genera and species of strigeid trematodes.

In general the topography of the genital ducts in the vicinity of the genital atrium is rather simple. The ventrally located uterus descends to the atrium as a thin walled, slightly modified or unmodified tube, and usually opens into the atrium at the base of a more or less conical papilla. The ejaculatory duct descends, just dorsal to the uterus, from the seminal vesicle to near the atrium where it usually discharges into the uterus, thus forming a hermaphroditic duct. The prostate normally descends into the genital papilla and opens into the atrium at the summit of the papilla. This simple arrangement of the genital ducts is sometimes greatly modified, and is always difficult to trace in whole mounts.

In the genus *Polycotyle* (Fig. 1, E) the uterus develops a small, though well outlined sphincter muscle before it is joined by the ejaculatory duct. The duct created by the fusion of these two ducts traverses only a short part of the genital papilla before fusing with the prostatic canal; the new duct penetrates the genital papilla to open into the atrium at its summit. In this genus, therefore, a slight change in the association of the genital ducts has been effected: the uterus no longer opens at the base of the papilla but joins the prostatic canal before discharging into the atrium.

As already pointed out, the association of the genital ducts in the material identified as *Crocodilicola pseudostoma* by us differs from that described by other workers for the same species. We find that the ejaculatory duct (Fig. 1, F) no longer discharges into the uterus but has become associated with the prostatic canal as is the case with the members of the genus *Pseudoneodiplostomum.* The uterus does unite with the

FIG. 1. Schematic representation of a probable evolutionary line of development of the prostate gland in the family PROTERODIPLOSTOMIDAE of the strigeid flukes. The type of development of the prostate together with its association with the other ducts of the genital tract may indicate the function of the organ. A = *Mesodiplostomum;* B = *Paradiplostomum, Herpetodiplostomum,* etc.; C & D = *Prolecithodiplostomum, Cystodiplostomum, Ophiodiplostomum* (?), etc.; E = *Polycotyle ornata;* F & F′ = *Crocodilicola;* G = *Pseudoneodiplostomum acetabulata;* H = *Pseudocrocodilicola americaniense;* I = *Pseudocrocodilicola georgiana;* J = *Pseudoneodiplostomum thomasi;* K = *Pseudoneodiplostomum siamense;* L = *Proterodiplostomum;* M = *Heterodiplostomum;* and O = *Pharyngostomum cordatum. Pharyngostomum cordatum* is included in the schematic diagram solely because of the evolutionary significance of its atrial recess.

prostatic canal and both discharge into the atrium through a common opening. It might be noted that the ejaculatory duct in the species *Pseudoneodiplostomum siamense* (Poirier, 1886) (Fig. 1, K) is surrounded by a mass of prostatic gland cells, the prostate being absent or only weakly developed. It will be remembered, the new species, *P. acetabulata* (Fig. 1, G), differs from the other two members of the genus *Pseudoneodiplostomum, P. thomasi* (Dollfus, 1935) (Fig. 1, J) and *P. siamense* (Poirier, 1886), in that the uterus joins the prostatic canal instead of opening separately from the prostatic canal as is the case with the other two species.

In the species *Pseudocrocodilicola americaniense* (Fig. 1, H) the ejaculatory duct joins the prostatic canal at about the middle of the prostate. The canal so formed discharges into the dilated part of the cavity of the secondary pouch, just dorsal to the opening of the uterus. In this species the proximal end of the uterus shows a modification of its wall in that the spongy-like tissue, mixed with muscle fibers, of the secondary pouch forms a short continuation of its tissue, and this surrounds the wall of the uterus. However, there is evidently no such continuation of the secondary pouch in the make up of the uterine wall in the area of the pouch in *P. georgiana* (Fig. 1, I). In this species, however, the ejaculatory duct joins with the prostatic canal at its extreme anterior end and both the uterus and prostate penetrate the wall of the secondary pouch to discharge simultaneously into the dilated portion of the cavity of the secondary pouch. A probable significance of the secondary pouch will be discussed later.

It is our opinion that the prostate gland is a definite, recognized feature of the anatomy within the family Proterodiplostomidae. Its nature and presence was suggested by Willemoes-Suhm (1870), Poirier (1886) and others but its true anatomy and possible significance perhaps was first indicated by Brandes (1890). Of the later workers, Dubois (1936b, 1938b) has done much to elucidate the details concerning the anatomy and relative connections of the prostate with the other genital ducts. As yet, however, the exact nature and function of the gland is more a matter of conjecture than an established fact.

Perhaps La Rue (1926a) came nearer to recognizing the way in which the prostate originated than had been previously indicated. In discussing the anatomy of *Pharyngostomum cordatum* (Diesing, 1850) (Fig. 1, O), this writer made this statement: "A slender recess (Fig. 5) extends from the genital atrium nearly to the seminal vesicle. A similarly situated structure is figured by Brandes (1890) in *Diplostomum abbreviatum* and in certain other forms, and by him is called the prostate gland. In the present species there is no indication of gland cells about this structure but there are well developed muscle fibers. I suspect that the prostate

described and figured by Brandes is not a prostate at all but a cavity of an eversible portion of the genital papilla." Whether or not an eversible portion of the genital cone has been demonstrated is more a matter of personal interpretation, although the existence of such a structure is highly probable. To us the prostate, as portrayed in this group of the strigeid flukes, undoubtedly represents a structure "on the way in" or one that is "on the way out." From an evolutionary point of view it does not matter which of the two alternatives one selects for argumentation. The slender recess of the genital atrium in *Pharyngostomum cordatum* undoubtedly represents simply a part of the genital atrium as is evidenced by the fact that its cavity is in open communication with the bursal portion of the atrium, and by the fact that the cavity is surrounded by a more or less dense mass of muscle fibers: such muscle fiber masses are present in tissue forming the bursa of all strigeid flukes known to us. However, the position of the recess, its length and its relation to the genital ducts would indicate its evolutionary significance. Should one be disposed to take the stand that the prostate is "on the way in," such a recess easily might represent the primordium of the gland. On the other hand, it might represent the vestigial remains of a prostate "on the way out." Certainly some such invagination as this recess formed at least the terminal part of the prostate. The cuticular lining of the central cavity of the gland would indicate such an origin.

Perhaps the invaginated recess of the prostate reaches its highest degree of specialization in the genus *Polycotyle*. Here the gland-like organ of the prostate (Fig. 1, E) has developed into an elongated, tubular structure with a heavy muscular wall and is surrounded by numerous, unicellular gland cells. These are rather large cells (Pl. II, Fig. 4) that show conspicuous nuclei and evacuolated cytoplasm. Each of the gland cells gives rise to a very fine duct that penetrates the musculature of the prostate to open into its lumen. The cells are more concentrated about the upper two-thirds of the prostate. The cavity of the gland is lined with a cuticular-like substance that becomes ridged to give it a corrugated appearance. It might be pointed out that the invagination of the prostate may become more extensively developed than is the case with the genus *Polycotyle*. In the genera *Prolecithodiplostomum* and *Cystodiplostomum* (Fig. 1, C & D) the prostate develops into a much elongated tubule and may extend forward as far as the caudal testis. However, in these genera the prostate appears to be a simple tube that does not reach the degree of specialization noted for the organ in *Polycotyle*. Such a degree of specialization is not attained in any of the other genera of the family with the possible exceptions to be found in the genera *Pseudocrocodilicola* and *Crocodilicola*. In the latter genus the cells about the prostate are much smaller and much less numerous, while in the former genus these

gland cells appear to be more like vitelline glands in comparison to the type of cell to be found in *Polycotyle*. The prostatic gland cells in the genus *Pseudocrocodilicola* appear to be filled with dark-staining granules and are more thinly dispersed in the parenchyma about the prostate and other genital ducts in the area.

As already suggested the prostate probably represents an invaginated part of the atrium. However, if the prostate, as it is recognized at the present time, is in any way comparable with any part of the cirrus pouch of the distomes, it is only feebly indicated in this group of the strigeids. The muscular nature of the organ, as well as its communicating gland cells, when these are present, would suggest that it is an accessory piece in the genital complex, and that it undoubtedly serves some ejaculatory or storage function. The ejaculatory function is made manifest when we consider those species in which the ejaculatory duct penetrates the musculature of the prostate to discharge into its cavity. Such an association is best exemplified in the species *Pseudoneodiplostomum thomasi* (Dollfus), *P. siamense* (Poirier), *P. acetabulata* n. sp., *Pseudocrocodilicola americaniense* n. sp. and *P. georgiana* n. sp. Should the condition described for the prostate and ejaculatory duct in *Pseudoneodiplostomum siamense* (Fig. 1, K) be established as correct, no other interpretation can be made but that the weakly muscular prostate with its numerous gland cells serves the ejaculatory duct in performing the function of discharging the male gametes. In this species the prostate is reduced to practically a mass of gland cells that surrounds the ejaculatory duct. In the species *Pseudocrocodilicola georgiana,* although the prostate is highly muscular but supplied with relatively few gland cells, the ejaculatory duct (Fig. 1, I) enters the prostatic canal at its anterior extremity. The spermatozoa, therefore, are discharged into the prostatic canal and must reach the outside only after traversing the entire length of the prostatic canal. In this species the prostatic canal and prostate frequently are seen to be greatly dilated and filled with male gametes, thus indicating that the prostate at least serves as a temporary storage space for the semen prior to ejaculation. Likewise, it is our observation that the prostatic canal in the other species already mentioned often become distended with spermatozoa. In these cases the musculature of the prostate must in some way serve as a pars prostatica in the expulsion of the male germ cells. We might add that the prostatic canal of *Polycotyle ornata* was not observed to contain spermatozoa but was filled with a fluid that had been coagulated by the fixing agent. In this species it will be remembered that the ejaculatory duct joined the uterus rather than the prostatic canal.

Perhaps the more primitive type of arrangement of the terminal portions of the genital ducts and their association with the prostate gland is best exemplified in the species *Paradiplostomum abbreviatum*. Here we

find a very small prostate (Fig. 1, B) that is confined to the genital papilla and bearing but very few gland cells. The prostate opens into the atrium at the summit of the papilla, while the ejaculatory duct fuses with the uterus and these two ducts discharge together into the atrium some distance from the opening of the prostate. It is quite likely that the more primitive condition would exclude the prostate entirely, as in *Mesodiplostomum gladiolum* Dubois (Fig. 1, A).

From the type of association as found in *Paradiplostomum abbreviatum* we are of the opinion that a dual migration of the uterus and ejaculatory duct has taken place. Along one line the uterus and ejaculatory duct have migrated together so that the short hermaphroditic canal formed by the fusion of these two ducts no longer opens directly into the atrium but fuses with the prostatic canal. Such a migration as this can be illustrated in *Polycotyle ornata* (Fig. 1, E) wherein the ejaculatory duct and uterus fuse into the formation of the hermaphroditic canal, and this in turn fuses with the prostatic canal. In this species only a single opening serves all of the genital ducts as well as the prostate. In pursuing this line further, the association found in the species *Pseudoneodiplostomum acetabulata* (Fig. 1, G) and *P. thomasi* (Fig. 1, J) presents evidence of a shift in the connection of the ejaculatory duct with the uterus to one in which that duct migrates across and becomes fused with the prostatic canal. This type of migration is perhaps perfected in *Pseudoneodiplostomum siamense* (Fig. 1, K) in which the ejaculatory duct penetrates the entire length of the reduced prostate. It is to be pointed out that the uterus in *Pseudoneodiplostomum thomasi* and *P. siamense* opens into the atrium independently of the prostatic canal. This may mean a lagging behind in the migration of the uterus or a backward shift.

The condition found in the genus *Pseudocrocodilicola* probably represents the full development in the shifting of the uterus and ejaculatory duct from an independent position to one of association with the prostate. In this genus the hermaphroditic canal has developed its musculature into what we have called a secondary pouch, and has become somewhat dilated into the formation of a genital cloaca. Very likely this development has taken place at the expense of the genital atrium for this formation is greatly reduced in the genus. Into the dilated cavity of the secondary pouch both the uterus and prostatic canal discharge. The ejaculatory duct in *P. americaniense* (Fig. 1, H) fuses with the prostatic canal at about the middle of the prostate, thus putting the prostate in line for a pars prostatic function. In the species *P. georgiana* (Fig. 1, I) such a function for the prostate is made more possible since the ejaculatory duct discharges into the prostatic canal at its anterior extremity.

Concerning the secondary pouch in the genus *Pseudocrocodilicola,* there appears to be no structure so far described among the strigeids that

is in any way comparable to it. In describing the species *Heterodiplostomum lanceolatum,* Dubois (1936b) described a cirrus pouch. The prostate is small but penetrates the cirrus pouch at its anterior end, and the prostatic canal ramifies the pouch before opening to the outside at the summit of the genital papilla. The association of the ejaculatory duct is with the uterus rather than with the prostatic canal, and these two ducts open into the atrium independently of the prostate. The association of the genital ducts and the presence of an eversible cirrus, an organ not observed in any of our material, in this species (Fig. 1, M) completely separates it from the material studied by us.

With the possible exception of the members of the subfamily POLYCOTYLINAE and the genus *Proterodiplostomum* all of the species described or studied by Dubois (1936b) exhibited an ejaculatory duct that joined the uterus to form a short hermaphroditic canal. This canal discharged into the genital atrium independently of the prostate. Likewise all of the species described by Dubois possessed a prostate with but one exception. The species *Mesodiplostomum gladiolum* (Fig. 1, A) is completely devoid of any structure that may be comparable with the prostate. The exact association of the genital ducts or the nature of the prostate, if such a structure exists, is not known for the species *Proalarioides serpentis* Yamaguti, 1933.

There is but little evidence pointing to the second line of evolution in this group of the strigeids. This line takes the direction of separating the uterus from the male genital duct and the prostate. In this group, represented by the *Proterodiplostomum* (Fig. 1, L), the uterus, ejaculatory duct and prostate may open separately into the atrium or the uterus opens into the atrium at the base of the genital papilla while the ejaculatory duct and prostatic canal open together at the summit of the papilla. As a matter of fact, this condition may represent an intermediate position between the pattern in *Paradiplostomum abbreviatum* and that displayed by *Pseudocrocodilicola georgiana.*

SUMMARY

The detailed anatomy is described for *Polycotyle ornata* Willemoes-Suhm, 1870, *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870), *Pseudoneodiplostomum acetabulata* n. sp., *Pseudocrocodilicola americaniense* n. sp. and *P. georgiana* n. sp. The last three named species are considered and described as new to science. All of the material considered by us came from the small intestine of the alligator, *Alligator mississipiensis* (Daudin).

The genus *Pseudocrocodilicola* is proposed as new. It is placed in the subfamily POLYCOTYLINAE Monticelli, 1888.

The evolution of the terminal portions of the genital ducts and the development of the prostate gland in the family PROTERODIPLOSTOMIDAE,

strigeid parasites of reptiles, is discussed with the view of throwing some light on the origin and function of the prostate gland.

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Explanation of Plates

Except for certain details of the terminal parts of the genital ducts all illustrations of the entire worm were made with the aid of the camera lucida. All illustrations of the posterior segment of the body are reconstructions made from serial sections.

Abbreviations Used

A—atrium
AS—atrial sucker
C—cecum
DS—dorsal sucker
EJ—ejaculatory duct
EP—excretory pore
GP—genital papilla
HD—hermaphroditic canal
LC—Laurer's canal
M—metraterm
MEJ—muscular portion of ejaculatory duct
O—ovary
OV—ova
P—prostate
PC—prostatic gland cells
SG—shell gland
SM—sphincter muscle of uterus
SP—secondary pouch
SR—seminal reservoir
SV—seminal vesicle
T_1—anterior testis
T_2—posterior testis
UT—uterus
VD—vas deferens
VE_1—vas efferentia of anterior testis
VE_2—vas efferentia of posterior testis
VIT—vitellaria
YD—yolk duct
YR—yolk reservoir

Plate I

Fig. 1. *Pseudoneodiplostomum acetabulata* n. sp., showing general contour of the body, the position of the various internal organs and the associations of the genital ducts with the prostate. Ventral view.

Fig. 2. *Pseudoneodiplostomum acetabulata* n. sp., showing reconstructed arrangement of the genital organs in the posterior segment of the body. The extreme posterior end of the body is made more detailed in order to show the associations of the genital ducts with the prostate, the length of the ceca, the metraterm and the posterior distribution of the vitellaria. Diagrammatic. Greatly enlarged. Lateral view.

Plate II

Fig. 3. *Polycotyle ornata* Willemoes-Suhm, 1870, showing general contour of the body, the position of the internal structures and the dorsal row of suckers. Anterior segment in ventral view. Posterior segment in lateral view.

Fig. 4. *Polycotyle ornata* Willemoes-Suhm, 1870, showing reconstructed arrangement of the genital organs and the associations of the genital ducts with the prostate. The posterior end of the body is shown in some detail so that the prostate, the prostatic gland cells, the sphincter muscle of the uterus, the union of the ejaculatory duct with the uterus, the atrial sucker, the termination of the cecum and the last two suckers of the dorsal row with the two very small suckers in between them could be made more clear. Diagrammatic. Greatly enlarged. Lateral view.

Plate III

Fig. 5. *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870), showing details of the body and the arrangement of the genital organs. Ventral view.

Fig. 6. *Crocodilicola pseudostoma* (Willemoes-Suhm, 1870) showing reconstructed view of the organs in the posterior half of the body. Note the size and shape of the prostate, the arrangement of the prostatic gland cells, the muscular wall of the ejaculatory duct and its connection with the prostate, and the muscular sphincter at the end of the uterus. Diagrammatic. Greatly enlarged. Ventral view.

Plate IV

Fig. 7. *Pseudocrocodilicola americaniense* n. g., n. sp., view of entire worm showing general contour of the body and the arrangement of the various internal organs. Ventral view.

Fig. 8. *Pseudocrocodilicola americaniense* n. g., n. sp., reconstruction of genital organs in posterior segment of the body. The extreme posterior margin of the body is represented more in detail in order to show the small atrium, the secondary pouch, the continuation of the pouch along the proximal part of the uterus, the prostate and its connection with the ejaculatory duct, the prostatic gland cells and the termination of the ceca. Diagrammatic. Greatly enlarged. Ventral view.

Plate V

Fig. 9. *Pseudocrocodilicola georgiana* n. g., n. sp., view of entire worm showing general contour of the body and the arrangement of the internal anatomy. Dorsal view.

Fig. 10. *Pseudocrocodilicola georgiana* n. g., n. sp., reconstruction of organization in the posterior segment of the body. Compare the anatomy of this species with that of *P. americaniense*. Diagrammatic. Greatly enlarged. Ventral view.

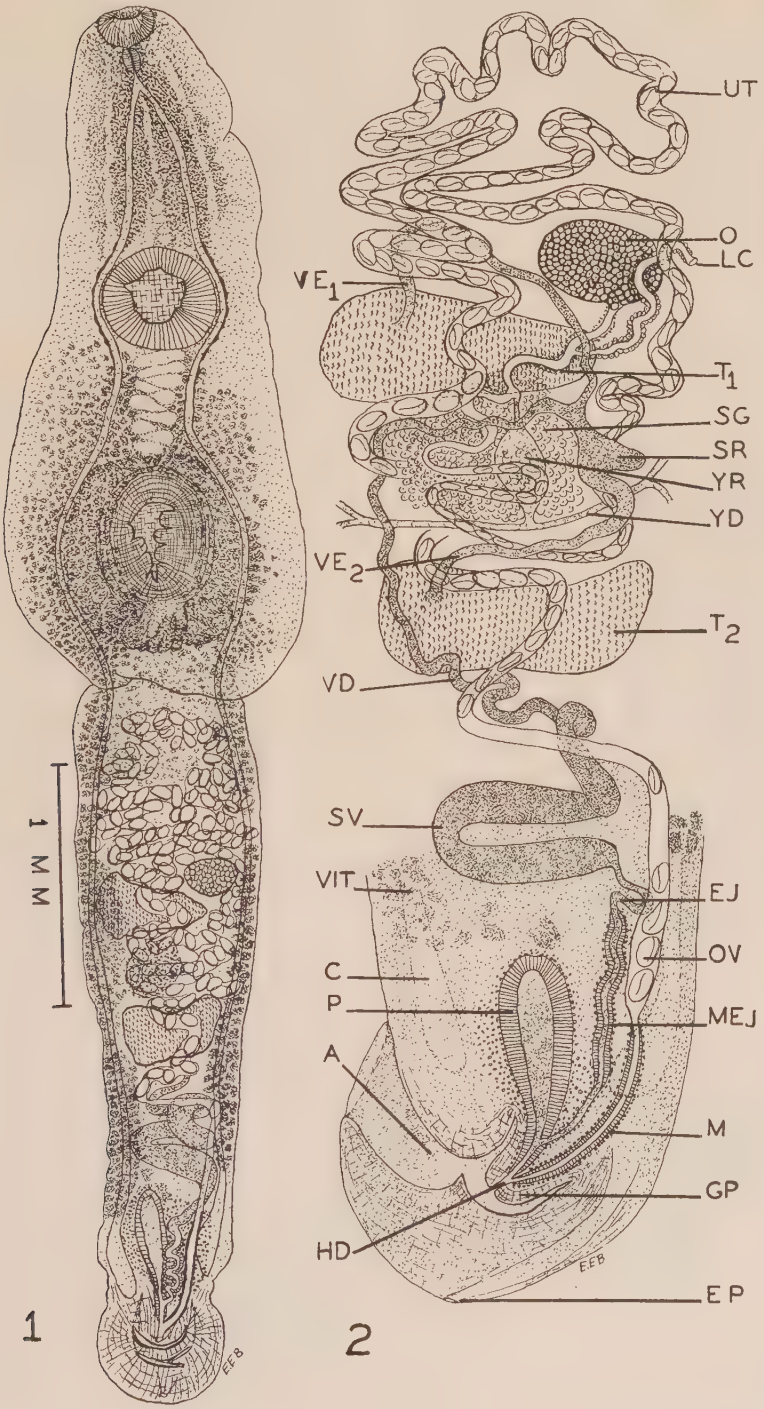
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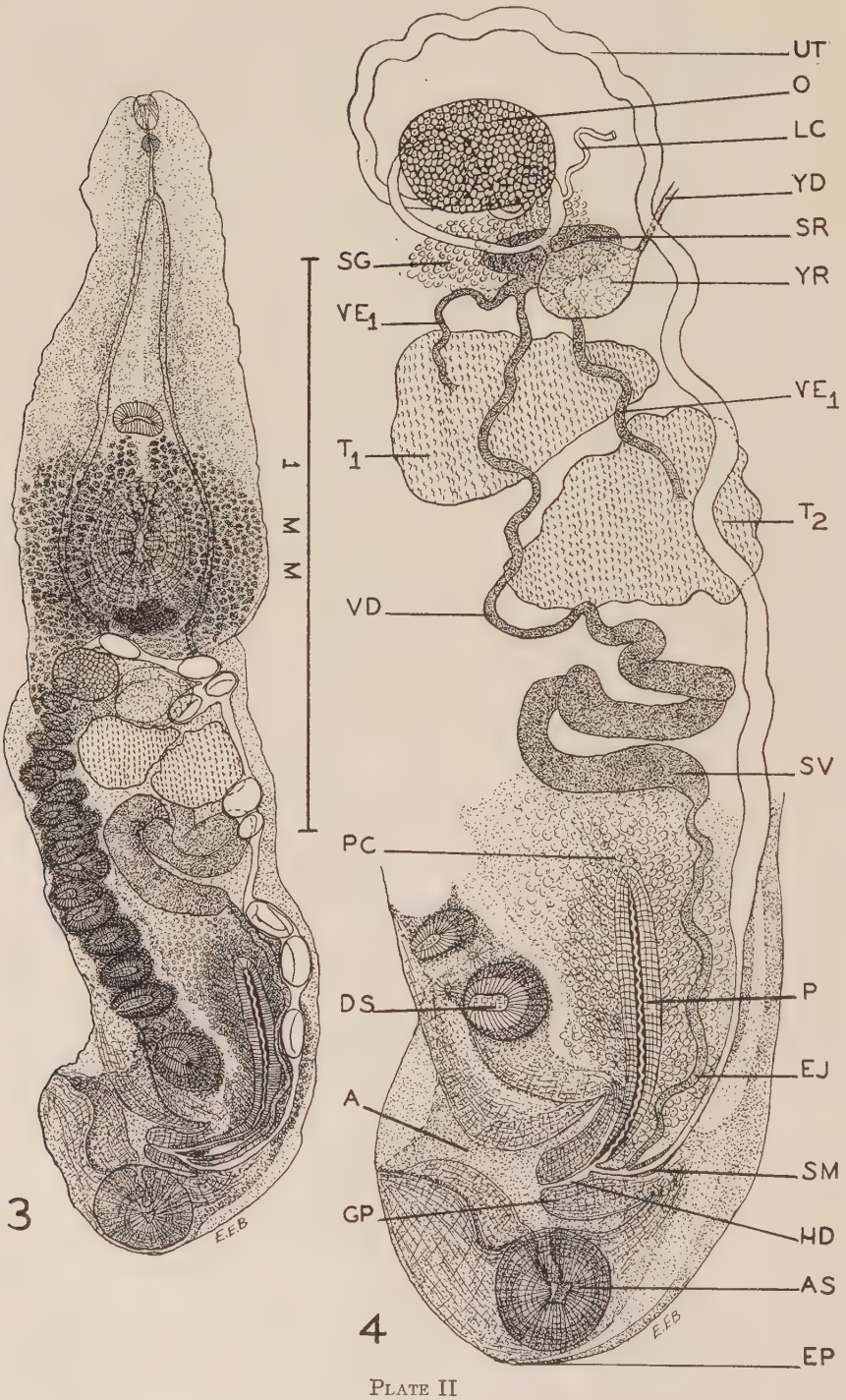
PLATE I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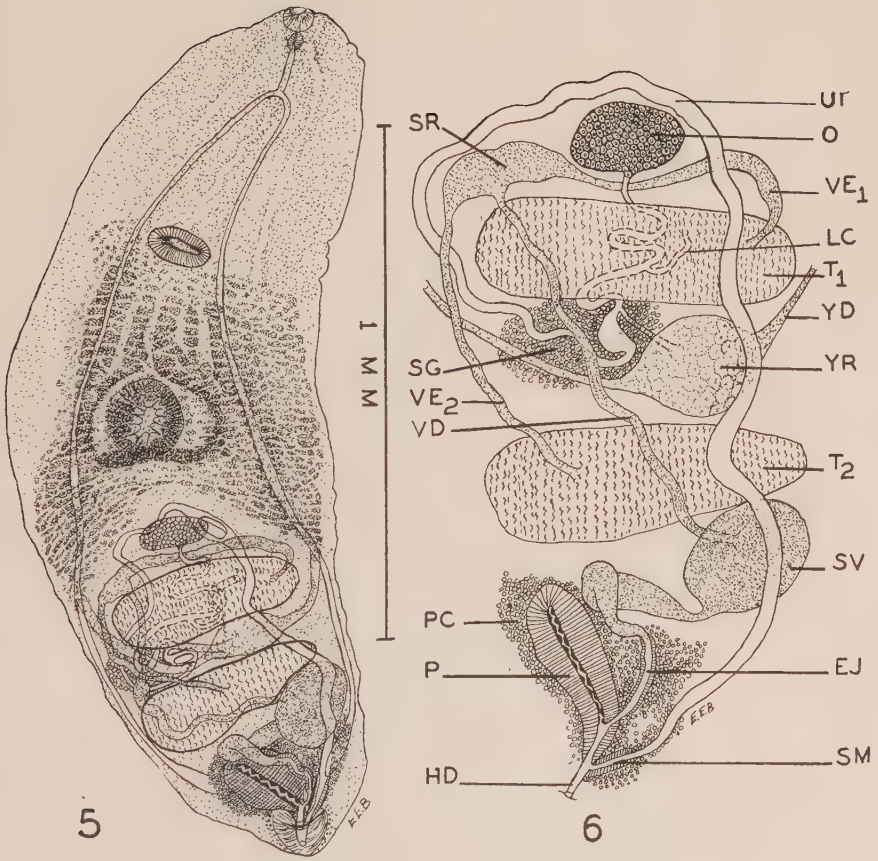

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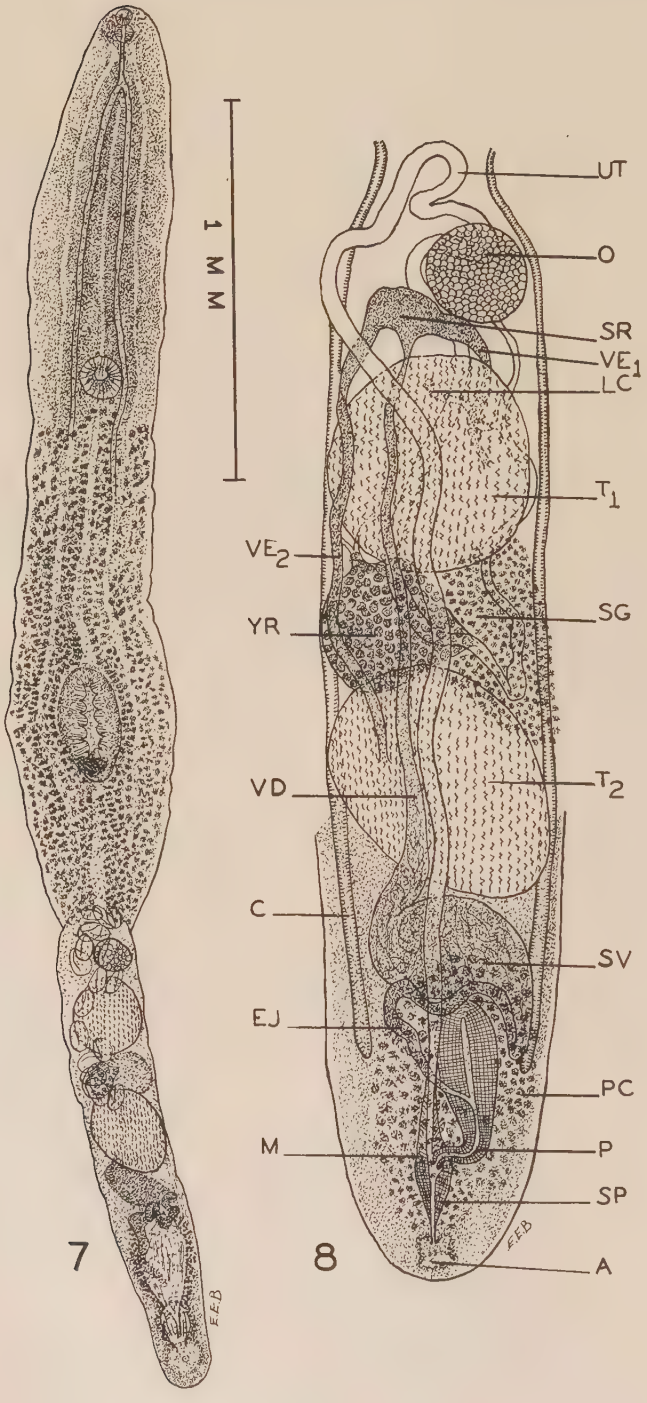

PLATE IV

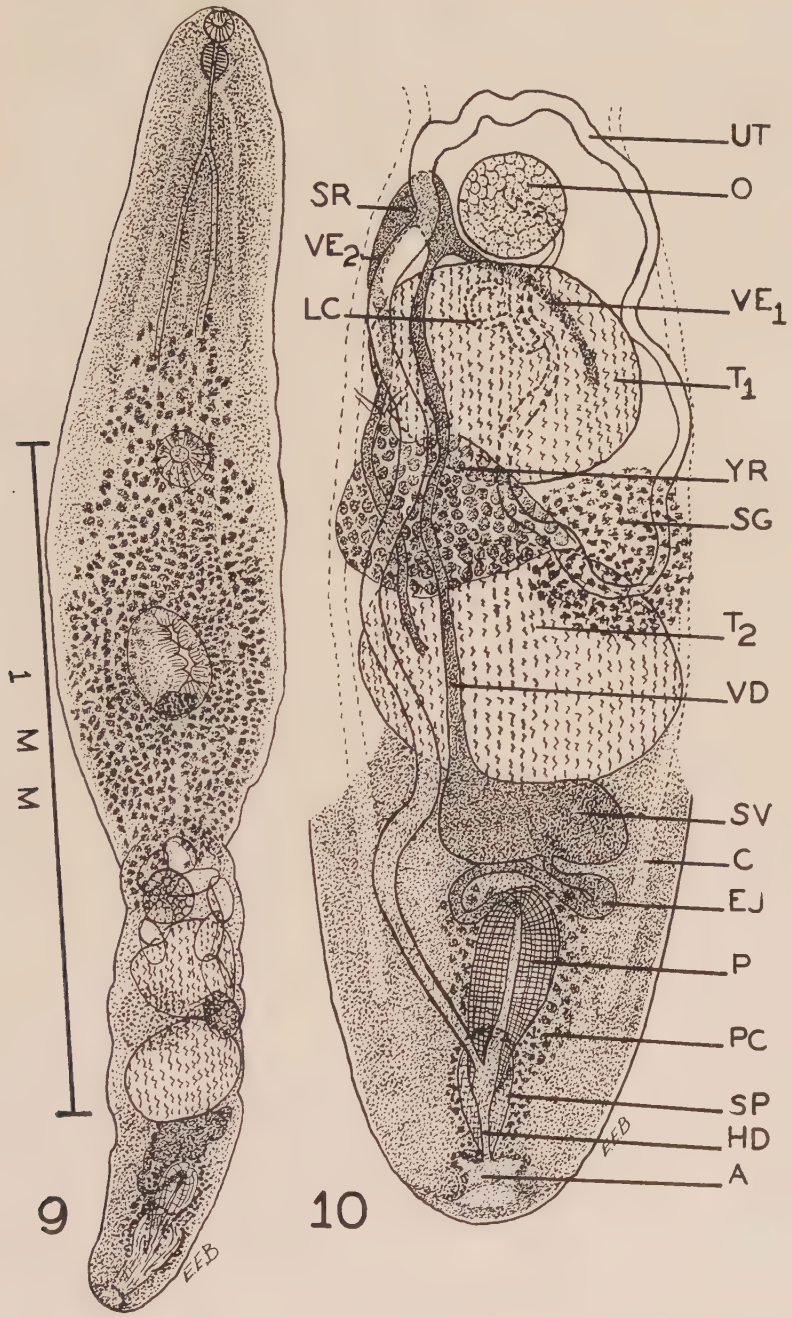

PLATE V

AN EXPERIMENTAL TEST OF THE LIFE CYCLE DESCRIBED FOR *COTYLURUS COMMUNIS* (HUGHES)*

Louis Olivier and W. W. Cort

On the basis of experiments conducted in 1926 and 1927, Van Haitsma (1930) presented a description of the life cycle of a strigeid trematode designated by him as *Cotylurus michiganensis*. Later, La Rue (1932) pointed out that the correct name for the species is *Cotylurus communis* (Hughes, 1928). Van Haitsma demonstrated by feeding experiments that strigeid metacercariae identified as *Tetracotyle communis* Hughes found on and about the hearts of common suckers, *Catostomus commersonnii* (Lacépède), became adults of *C. communis* in the bursa Fabricii of the herring gull, *Larus argentatus* (Pont.). He then collected eggs from the bursae of the experimentally infected gulls and allowed them to embryonate. Seven species of snails were exposed to the miracidia which developed. One of the snails, a specimen of *Lymnaea emarginata angulata* Sowerby (= *Stagnicola emarginata angulata*), produced large numbers of strigeid cercariae forty days later. This snail had been collected from Douglas Lake and kept in the laboratory three weeks before it was exposed to the miracidia. The intestine of the gull, from which the eggs used in this particular experiment were obtained, harbored two specimens of a species of *Diplostomum*. The bursa contained many specimens of *C. communis*. Van Haitsma concluded that the single snail mentioned had been infected experimentally and that the cercariae belonged to *C. communis*. He stated that similar experiments conducted in 1928 yielded the same results but he did not describe these experiments.

Cort and Brackett (1937) considered the cercariae obtained by Van Haitsma to be identical with *C. emarginatae* Cort, 1917 which was described from the same host in the Douglas Lake Region.

Szidat (1931, p. 166) questioned the validity of the life cycle of *C. communis* as outlined above. He believed that the location of the penetration glands in the cercariae of members of the same genus should be similar. Since the penetration glands of Van Haitsma's cercaria lie behind the ventral sucker while those of the cercaria of *C. cornutus* are in the front of the ventral sucker, he felt that the two species were prob-

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ably not closely related. Consequently, he suggested that Van Haitsma's cercaria might belong to the species of *Diplostomum* that was in the intestine of the bird from which the eggs used in the experiment were taken. Dubois (1938) also questioned the validity of this part of the life cycle. He noted that the snail in which the cercariae appeared had been in the laboratory only three weeks before exposure to the miracidia. This seemed to him an insufficient time to preclude the possibility of previous infection.

Since these workers had seriously questioned the correctness of the life cycle described for *C. communis,* it seemed desirable to throw further light on the matter. Therefore, we attempted to complete experimentally that part of the cycle not carried through by Van Haitsma, namely, the infection of the second intermediate host with the subsequent recovery of the mature metacercariae. If *Tetracotyle communis* could be recovered from fish after they had been exposed to Van Haitsma's cercaria, the doubt concerning the validity of the described life cycle would be removed.

INFECTION EXPERIMENTS

The cercaria described by Van Haitsma (1930) as belonging to *C. communis,* and originally described as *C. emarginatae,* is common in the Douglas Lake region. Its structure is very characteristic and it can be readily distinguished from all other strigeid cercariae which occur in the region. Several specimens of *S. emarginata angulata* harboring infections with this cercaria were used as the source of the cercariae for the infection experiments. In order to make certain that these cercariae were identical with those of Van Haitsma, their morphology was worked out in detail and checked against his description. No differences were discovered, but certain additional morphological information was secured which will be given later. We are certain, therefore, that the cercariae which we used in the following infection experiments belonged to the same species as those which Van Haitsma described as belonging to *C. communis.*

In a preliminary experiment two perch (*Perca flavescens* (Mitch.)) from Vincent Lake were exposed to these cercariae. Vincent Lake is an acid lake which harbors no snails and no trematodes have ever been found in fish taken from it. The two perch were placed in an aquarium supplied with a small stream of lake water and water containing cercariae was added on July 25, 1940. On July 27, one of the fish was dissected and metacercariae with all the characteristics of the cercarial body were recovered from both eyes. Additional cercariae were added to the aquarium on August 1, 12, and 20. The second fish was examined on August 22 and numerous metacercariae of the diplostomulum type in four distinct stages of development were recovered from both eyes. It was evi-

dent, therefore, that the metacercariae recovered had been introduced experimentally and were 2, 10, 21, and 28 days old. Three perch of the same collection were maintained under identical conditions in another aquarium but were not exposed to cercariae. No metacercariae were found in these fish on August 22.

Subsequently, we conducted three additional experiments involving a total of fourteen perch from Vincent Lake. Cercariae were introduced into the tanks periodically as in the above experiment. Metacercariae were recovered from the eyes of all the fish after from two to 26 days. In one of the experiments only well water was used to preclude the possibility of contamination from the lake water.

One bull-head (*Ameiurus nebulosus* (LeSueur)), five blue-gills (*Lepomis macrochirus* (Rafinesque)), four rock bass (*Ambloplites rupestris* (Rafinesque)), and three log perch (*Percina caprodes* (Rafinesque)) were also exposed to the cercariae but none became infected.

The results of these experiments demonstrate that the cercaria described by Van Haitsma as belonging to *C. communis* penetrates into perch and develops in the eyes into a typical diplostomulum. Therefore, it can have no relationship to the life cycle of *C. communis* since the metacercaria of this species is a tetracotyle found around the hearts of fishes. This finding indicates that Szidat and Dubois were correct in questioning the validity of Van Haitsma's experiments. Either the snails he used were previously infected or were infected with miracidia from the eggs of diplostomes present in the intestine of his gull host. Therefore, the species of cercaria which Van Haitsma (1930) mistakenly connected with *C. communis* should now be known as *Cercaria emarginatae* Cort, 1917.

DATA ON *C. emarginatae* AND ITS METACERCARIAL STAGE

The original description by Cort (1917) of *C. emarginatae* is very incomplete but Van Haitsma's later description is given in considerable detail. As noted above, a careful study of the cercariae used in our experiments failed to reveal differences between his description and our material. However, we are able to add a few details to the description as a result of our studies. We will also include descriptions of the immature and mature metacercariae which we obtained from the experimentally infected fish, followed by information we have gathered concerning the host-parasite relations of the metacercariae.

Additions to the description of C. emarginatae. The arrangement of the excretory tubules, not determined by Van Haitsma (1930, fig. A), appears in Fig. 1. The proximal portion of the posterior collecting tubule bears three patches of cilia. The ventral body spination is shown

in Fig. 1. About nine transverse rows of spines occur in the region between the circum-oral band of spines and the level of the ventral sucker. Scattered spines occur among the first three or four transverse rows. The ventral surface behind the acetabulum is covered with scattered spines except for a small median spineless area. The dorsal body spination is identical except that no spines occur posterior to the level of the ventral sucker and the spines at the level of the pharynx are more sparse. Six to nine hooked "forward projecting spines" always appear

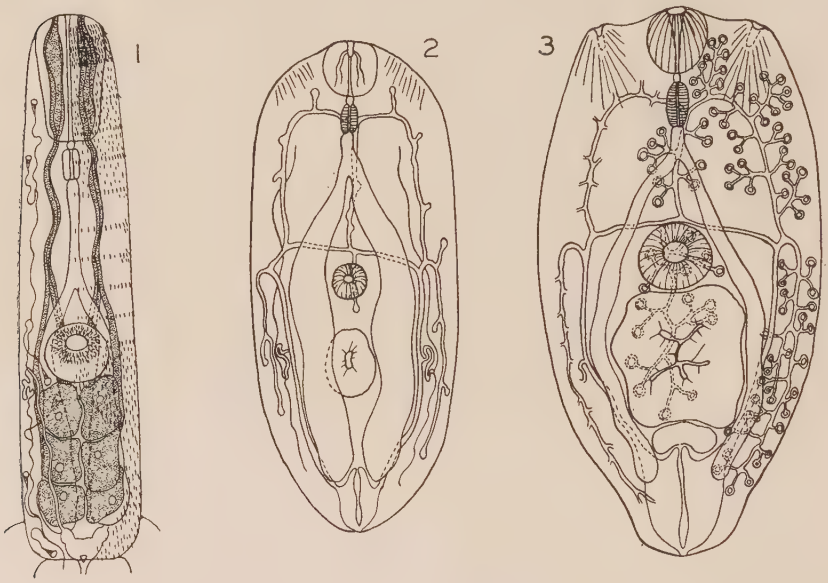

FIG. 1. Body of *Cercaria emarginatae* to supplement Fig. A in Van Haitsma (1930). Ventral aspect. Excretory system is shown only on the left; spination only on the right.

FIG. 2. Developing metacercaria (10-day-larva) of *C. emarginatae* from eye of perch. Ventral aspect. Contents of ceca not shown. Note the developing reserve excretory system, small holdfast, and remnants of gland ducts in the partially metamorphosed oral sucker.

FIG. 3. Mature metacercaria (28-day-larva) of *C. emarginatae* from eye of perch. Ventral aspect. To the same scale as Fig. 2. Reserve excretory system is incompletely shown on left.

immediately dorsal to the oral opening. The spines on the ventral sucker and in the circum-oral band are also hooked. The intestinal ceca are very prominent and the esophagus and portions of the ceca lying anterior to the acetabulum are usually expanded so that under low magnification there appears to be a large clear area in front of the ventral sucker. Twenty-five specimens fixed by the usual method (Cort and Brackett, 1937) were measured and the results are as follows:

	Range (mm)	*Mean with P.E. (mm)*
Body length	0.221–0.268	0.253 ± 0.002
Body width	0.040–0.047	0.046 ± 0.001
Tail stem length	0.308–0.368	0.344 ± 0.002
Tail stem width	0.034–0.047	0.040 ± 0.001
Furcal length	0.288–0.335	0.315 ± 0.002
Oral sucker length	0.056–0.069	0.062 ± 0.001
Oral sucker width	0.021–0.027	0.024 ± 0.001
Ventral sucker diameter	0.024–0.029	0.027 ± 0.001

Description of immature metacercariae. Larvae recovered two days after penetration retained all the characteristics of the cercarial body and some of the details of the cercarial structure were checked by studying them. It is noteworthy that in these young larvae the excretory system and spination were unchanged and only a small part, if any, of the contents of the penetration glands had been lost. The larvae reached the eyes in large numbers within two days after penetration.

Larvae ten days old were obviously immature but had undergone considerable morphological change (Fig. 2). The oral sucker was partially changed though the gland ducts in it could still be seen. The main channels of the reserve excretory system had developed and some of the smaller branches had appeared. The holdfast was small but had a lumen. Lateral sucking areas were beginning to develop on either side of the oral sucker. The digestive ceca were very conspicuous since they were large and contained much black pigment in the form of small rods and granules. The hind body was just beginning to form. Thirteen worms fixed at this stage were 0.36–0.44 mm long and 0.11–0.20 mm broad. Thirteen and fourteen day old metacercariae were still very similar to the ten-day larvae.

Larvae twenty-one days old had almost completed their metamorphosis. The only indication of morphological immaturity was that the excretory concretions were noticeably smaller than those of older metacercariae.

Description of mature metacercariae. The oldest larvae secured in the experiments were twenty-eight days old. They had all the characteristics of typical diplostomula and were apparently mature morphologically (Fig. 3). The forebody was flat and broad. The holdfast organ was large, filling the space between the ventral sucker and the excretory bladder. The lateral glandular areas which lie on either side of the oral sucker were conspicuous and were usually protruded slightly in living specimens. In fixed specimens they often appeared as cup-like lateral depressions. The form and extent of the reserve excretory system are shown in Fig. 3. The concretions at the ends of the channels varied from 3 to 12 microns in diameter and were usually spherical although some were oblong or irregular. The intestinal ceca contained no pigment and were relatively narrow and inconspicuous. Numerous,

small gland-like structures were observed beneath the cuticula on the anterior two-thirds of the worms. Fifteen fixed specimens were 0.37–0.49 mm long and 0.14–0.25 mm broad. The diameter of the oral sucker in ten specimens ranged from 0.036 to 0.041 mm and the diameter of the ventral sucker from 0.038 to 0.050 mm. The pharynx in seven fixed specimens was 0.036 to 0.043 mm long and 0.019 to 0.024 mm broad. Although it is not expected that older metacercariae of this species will be found to differ appreciably in structure, they may be larger than the metacercariae just described, since as pointed out by Cort and Brackett (1938), strigeid diplostomula may increase in size for some time after reaching apparent maturity. It may also be added that some strigeid larvae are not infective when they reach what may be called morphological maturity but require an additional period during which no evident morphological changes occur (Olivier, 1940, p. 456).

Location of metacercariae in the second intermediate host. As stated previously, dissection of experimental fish always revealed that the metacercariae develop exclusively in the eyes. Eyes containing metacercariae of different ages were fixed and sectioned. Study of this material revealed that almost all of the metacercariae were located in the retina between the sensory cells and the pigmented layer. Only two were found elsewhere; one was in the optic nerve near its exit from the eye ball, and the other in the layer of ganglion cells of the retina.

Damage to the retina was usually limited to the sensory cells immediately adjacent to the parasites. One of the eyes which harbored two-day-larvae had a large quantity of blood in a space apparently created by the parasites between the pigmented layer of the retina and the layer of sensory cells. In the other eyes there was no indication of hemorrhage or of a defense reaction to the parasites.

Evidently the young metacercariae feed on the tissue of the retina since the ceca of the ten, thirteen, and fourteen day larvae were filled with black material which was obviously retinal pigment. The ceca of older larvae, twenty-one to twenty-eight days after penetration, contained no pigment. It seems likely that these older larvae had stopped actively ingesting food and probably were securing their nourishment by osmosis through the body wall since their ceca were smaller than those of the younger larvae and were free from the pigment.

Most of the larvae were found near the margins of the retina although some were at other levels including that portion immediately behind the lens. Consequently, it is probable that even moderately heavy infections in perch do not seriously impair vision. Experimentally infected perch harboring large numbers of worms did not seem to be adversely affected.

RELATIONSHIPS

C. emarginatae and its metacercaria are strikingly similar morpho-

logically to the corresponding stages in the life cycle of *Diplostomum flexicaudum*. These similarities must indicate that the two species are closely related and it seems reasonable to predict that the adult of *C. emarginatae* is a member of the DIPLOSTOMIDAE and is probably in the genus *Diplostomum*.

SUMMARY

Infection experiments have demonstrated that the cercaria described by Van Haitsma (1930) as the cercaria of *Cotylurus communis* does not belong to that species. These cercariae, for which the name *Cercaria emarginatae* Cort, 1917 is brought forward, penetrate perch (*Perca flavescens*) and develop in the eyes to typical, unencysted diplostomula similar to those of *Diplostomum flexicaudum*. The metacercariae of *C. communis* are known to be encysted tetracotyle larvae which occur principally about the hearts of fishes. Additions are made to the description of *C. emarginatae,* and the metacercariae into which it develops are described. The metacercariae develop almost exclusively in the retina between the pigmented layer and the layer of sensory cells. When the larvae are young they evidently feed on the tissue of the retina since they contain large quantities of pigment. Since most of the larvae occur in the peripheral portions of the retina and do not cause extensive damage, they probably do not interfere seriously with vision unless present in very large numbers.

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ON TWO NEW SPECIES OF MYXOSPORIDIA FROM ILLINOIS FISHES*

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During the spring and summer of 1935, a number of Illinois fishes were autopsied. In a previous publication (1937) the writer presented a summary of the Myxosporidia found during the study, including the record of a number of forms identified to genus but not speciated. Two of these were restudied and are described in this paper.

All of the fishes were taken in the Ohio River and shipped to Urbana, Illinois, on ice. The material was studied as rapidly as possible in fresh condition. In most cases the Myxosporidia were still alive and motile during the preliminary observations. The parasites were preserved in 4% formaldehyde and have been studied recently in stained and unstained preparations. Permanent slides were stained with Heidenhain's iron haematoxylin or Giemsa.

Chloromyxum opladeli n. sp.

(Figs. 1–9)

Habitat. This myxosporidian occurs in the gall bladder of *Opladelus olivaris*. Two of twenty fishes examined were infected, the gall bladder of one of which also contained *Myxidium* sp. These fishes were obtained from the Ohio River at Shawneetown and Rosiclaire, Illinois.

Trophic stage. One host contained large numbers of active trophozoites; the other contained many mature spores but few vegetative forms. Immature trophozoites were small, with about 10 μ the minimum diameter. Mature forms with developing spores measured from 18 to 30 μ in diameter. The hyaline ectoplasm formed a broad girdle about the oval, somewhat flattened organism. The granular endoplasm contained many small refractive inclusions. Many nuclei, visible in vivo, stained deeply with basic dyes. Developing pansporoblasts and mature spores were found in the alveolar endoplasm.

Spore. The majority of spores were typical of the genus *Chloromyxum*. They were subspherical in front and side views and contained four polar capsules at the anterior end of the spore. A rather prominent, slightly elevated, sutural ridge, visible in both fresh and stained spores, was present (Figs. 2, 3). In some cases the sutural ridge was

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slightly curved. Each valve bore up to eleven distinct striae which converged anteriorly. The sporoplasm was small and filled the extracapsular cavity of the spore. Two small nuclei were found, occupying a somewhat excentric position in the majority of spores (Fig. 4). The polar capsules were pyriform, converging slightly, but opening separately. Small, flattened capsulogenous nuclei were persistent, and could be seen in a pycnotic condition in most of the stained spores. The polar capsules were approximately equal in size. Spore dimensions were: length, 6.5–8 (7.4) μ; breadth, 6–7.5 (6.7) μ; thickness, 5.5–7 (6.3) μ; capsules 2.5–3 (2.8) μ by 1.5–2.3 (1.9) μ.

Atypical spores, with two, six or eight polar capsules, were quite numerous. In the two hosts those with six polar capsules comprised 30% and 25% of all spores present. In their major morphological characters they resembled the typical spores, but were somewhat larger, and the polar capsules were somewhat smaller. There were three shell valves, fitting together along three distinct sutural ridges (Fig. 5). In anterior view the spore had a rather triangular shape. Striations similar to those found on typical spores ran more or less parallel to the sutural ridges. The polar capsules were pyriform and relatively small. Each bore a small flattened, pycnotic capsulogenous nucleus (Fig. 6). The sporoplasm was large and contained three nuclei. These spores averaged about 8.3 μ in length; 7.5 μ in breadth; 7.4 μ in thickness; capsules 2.5 by 1.6 μ.

Relatively small numbers of spores with two polar capsules occurred. 5% and 3% of all spores were of this type in the two hosts. These spores were smaller than typical spores but had few distinctive characteristics. The spore membrane was thin, and there was a slightly elevated sutural ridge (Fig 6). Seven to nine striae were present on each valve, converging anteriorly. The average dimensions of the few spores of this type were: length, 6.3 μ; breadth, 6.0 μ; thickness, 5.8 μ; capsules 2.5 by 1.9 μ.

Occasionally spores with eight polar capsules were found. The morphology of these is not well known because of the small numbers in which they occurred (about 1% of all spores in each host). The spore membrane was composed of four valves meeting along low sutural ridges (Fig. 9). Striae, converging anteriorly, were present on each valve. A typical spore of this type measured: length, 8.4 μ; breadth, 7.6 μ; capsules, 2.5 by 1.2 μ.

Discussion. Atypical spores are not rare among Myxosporidia. They have been described in a number of species belonging to several genera and probably are more widespread than the literature might suggest as they usually occur in relatively small numbers and present no important morphological problem. Aberrations of various types are

known. One of the commonest forms of aberrancy is the appearance of short caudal processes on spores of *Myxobolus* or *Myxosoma,* as, for example, *Myxobolus hylae* (Johnston and Bancroft, 1918), *Myxobolus mulleri* (Bütschli, 1881), *Myxosoma dujardini* (Thélohan, 1895) and *Myxosoma funduli* (Kudo, 1918). A change in the size or position of polar capsules may produce abnormal asymmetrical spores, as in *Myxobolus aeglefinis* (Auerbach, 1906), *Myxobolus orbiculatus* (Kudo, 1920), and *Mitraspora elongata* (Kudo, 1920). An aberration involving the appearance of a supernumerary polar capsule occurs in *Chloromyxum catostomi* (Kudo, 1920). Although such atypical spores have an altered morphology there is little effect on the major axes of the spore. The writer is familiar with but few instances in which the aberrancy involves a readjustment of the axial arrangement of the spore. Noble (1939) mentions and figures spores with four polar capsules which he found frequently in fishes infected with *Trilospora californica.* The spores are normally characterized by tri-radiate symmetry but spores with four capsules are tetra-axiate. This is especially interesting since the typical spores resemble a tri-radiate *Ceratomyxa.*

The present form is unusual, then, in having such a high frequency of aberrant spores, and in the extent to which the aberrancy effects the axis of the spore. The bilateral symmetry of the typical *Chloromyxum* spore is supplanted by tri-radiate symmetry in spores with six polar capsules, and tetra-radiate symmetry in spores with eight polar capsules. Whether such aberrancies represent a response on the part of the parasite to an unfamiliar host, similar to that observed by Bond (1937) in *Myxosoma grandis,* or are the result of some internal factor, is not yet known.

The mode of formation of aberrant spores presents several interesting problems. Any one trophozoite may produce several kinds of spores. Fig. 1 shows a trophozoite containing one spore with eight polar capsules and two lying together with two and six polar capsules. Each pansporoblast must give rise to four sporoplasmic nuclei, four nuclei associated with the spore membrane and eight capsulogenous nuclei, distributed equally to the two spores formed, if development is typical. In many myxosporidia two residual nuclei remain in the pansporoblast. In the formation of atypical spores the distribution of nuclei to the spores is modified. A pansporoblast can give rise to one spore with eight polar capsules or two spores with two and six polar capsules respectively. In the spore with eight polar capsules, all of the nuclei are given to the single spore. Each polar capsule has one capsulogenous nucleus. The sporoplasm in some spores studied carried four nuclei. Unfortunately, so few developing spores of this type have been found that it is not possible to determine whether the usual relationships of valves to shell nuclei prevail, but it

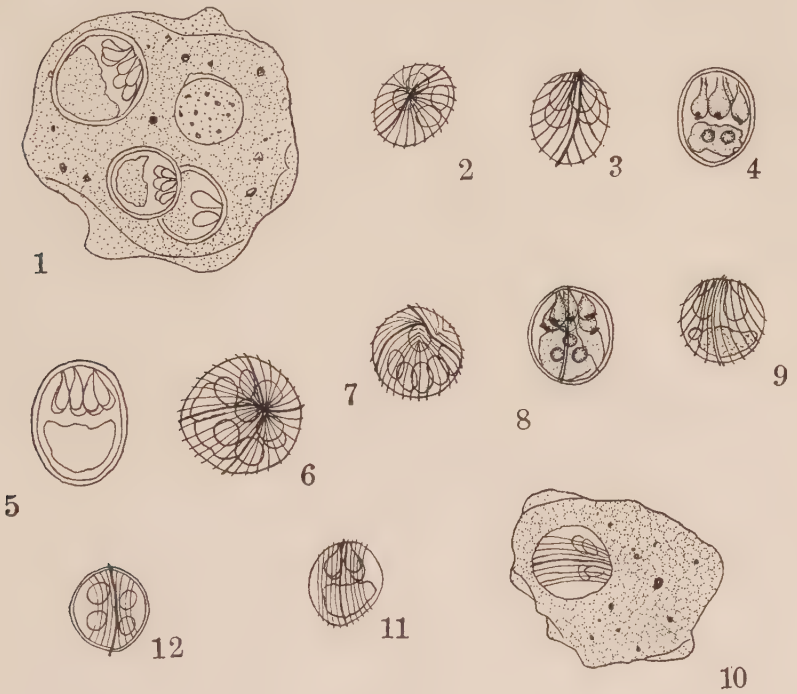
seems probable that the four valves are each accompanied by a single nucleus. Thus all of the nuclei of the pansporoblast are accounted for.

Spores with two polar capsules and six polar capsules developing from a pansporoblast present a discrepancy in the number of nuclei. The eight capsulogenous nuclei are accounted for, six being given to one spore, two to the other. The sporoplasmic nuclei are likewise accounted for since there are three in the spore with six polar capsules and one in the spore with two polar capsules. In nuclei associated with the spore membrane, however, there is a discrepancy. In typical development, four such nuclei are formed, two going to each spore, which, in consequence is bivalve. But in this type of atypical development the six-capsuled spore has three valves and the bicapsular spore has two valves. Thus five valves develop from the pansporoblast. Preliminary observations have shown that there are three shell nuclei in the developing spores with six polar capsules, and several spores with two polar capsules have been found which contained two shell nuclei, one associated with each valve. This extra nucleus cannot come from capsulogenous nor sporoplasmic nuclei since these are all accounted for. Thus one of the valve nuclei may undergo an extra division, or one of the residual nuclei of the pansporoblast may be used. It has not been determined which of these takes place.

According to the observations made to the present time the spores with six polar capsules develop from a pansporoblast which gives rise to another spore with two polar capsules. The two types of spores are seen lying side by side in trophozoites not infrequently (Fig. 1). When floating free in the bile, however, a high percentage of spores with six polar capsules are found (25–30%) and a very low percentage with two polar capsules (3–5%). If the two develop together there should be an equal number of each. Although a few sporoblasts developing into single typical spores have been seen, none developing into spores with six polar capsules have been found. Even though some may develop in this way, it cannot account for the whole discrepancy since they must be quite rare. The inference is drawn that spores with two capsules rarely complete their development or degenerate in the bile soon after their formation.

Identification. Of the 41 species of *Chloromyxum* which are listed in Kudo (1933), none have characteristics which can be considered identical with the present form. Of the various species described by various investigators those most closely resembling the present form, exclusive of the formation of atypical spores, are *Chloromyxum fluviatile* Thélohan, *Chloromyxum truttae* Légèr, *Chloromyxum thymalli* Lebzelter, *Chloromyxum misgurni* Kudo, and *Chloromyxum granulosum* Davis. *Chloromyxum fluviatile* is differentiated by the posterior points

shown on the shell valves (Thélohan, 1892 discussed by Kudo, 1920). *Chloromyxum truttae* has a slightly different spore shape and has trophozoites either elongate or without pseudopodia (Légèr, 1906).



Figs. 1–12

All figures are camera lucida drawings magnified 1590 times. Unless otherwise stated the spores are preserved in 10% formalin and unstained.

Chloromyxum opladeli

1. Trophozoite with atypical spores.
2. Typical spore, oblique view.
3. Typical spore, side view.
4. Typical spore, front view. Heidenhain's haematoxylin.
5. Spore with six polar capsules.
6. Spore with six polar capsules. Heidenhain's haematoxylin.
7. Spore with two polar capsules, side view.
8. Spore with eight polar capsules.
9. Spore with eight polar capsules.

Chloromyxum thompsoni

10. Trophozoite containing a young spore.
11. Spore, side view.
12. Spore, anterior view.

Chloromyxum thymalli may be distinguished from the present form by its tendency to produce six spores in each trophozoite, and the somewhat wavy valve striations (Lebzelter, 1912). *Chloromyxum misgurni*

differs from the present form by its habit of attaching to the wall of the gall bladder in the trophic stage, the failure of the sutural lines to converge anteriorly, and the straight sutural line (Kudo, 1916). *Chloromyxum granulosum* is differentiated by the small number of striations on the spore valves (Davis, 1917). As a result, this form has been considered as a new species, and the name *Chloromyxum opladeli,* is proposed for it.

Chloromyxum thompsoni n. sp.

(Figs. 10–12)

Habitat. This organism occurs in the gall bladder of one *Ictiobus bubalus* of three examined. All of the fishes were from the Ohio River at Shawneetown, Illinois.

Vegetative form. A few trophozoites bearing spores were found. These were small amoeboid forms measuring from 15 to 25 μ in diameter (Fig. 10). There was no distinct ectoplasm and movement was very sluggish. Probably the trophic stages were somewhat abnormal when first examined. In stained trophozoites many small nuclei were found in the granular, alveolated endoplasm. Pansporoblasts and developing spores were numerous. The trophozoites are usually polysporous, containing from two to four pansporoblasts in different stages of development.

Spore. The spore was small and subspherical, with rather thick valves and a conspicuous sutural ridge. Each valve bore about five well developed, distinctly elevated striations which extended from the anterior to the posterior end of the spore. They lay parallel to the sutural ridge, and covered the median half of the spore membrane (Figs. 11, 12) in side view. The faces of the valves carried no striae. Four large, broadly pyriform polar capsules occupied the anterior half of the spore. The capsulogenous nuclei were not found in mature spores. Valve nuclei were also lost before the spore became mature. Two small sporoplasmic nuclei lay in the sporoplasm which occupied the posterior half of the spore cavity. The sporoplasm was usually indented at the base of the capsules. Spores measured approximately 6 to 8 (6.9) μ in length, 6 to 7.5 (6.5) μ in breadth, 5.5 to 7 (6.3) μ in thickness. Capsules measured 2 to 3 (2.6) μ by 1.5 to 2 (1.6) μ.

Identification. The species which most closely resemble the form under consideration are *Chloromyxum fluviatile* Thélohan, *C. granulosum* Davis and *C. catostomi* Kudo. *C. fluviatile* differs from the present form by having three or four fine points on the posterior half of the spore (Thélohan, 1892, discussed by Kudo, 1920). *C. granulosum* differs in having striae which converge anteriorly (Davis, 1917). *C. catostomi* is most similar, but differs in having trophozoites with filiform pseudopodia attached to the wall of the gall bladder, more rounded polar

capsules, and very fine striations instead of relatively coarse ones as found in the present form (Kudo, 1920). No atypical spores with five capsules similar to these described in *Chloromyxum catostomi* have been found. For these reasons the present form is believed to be an undescribed species, and the name *Chloromyxum thompsoni* is proposed for it.

SUMMARY

1. Two new species of *Chloromyxum* from Illinois fishes are described, *Chloromyxum opladeli* n. sp. from the gall bladder of *Opladelus olivaris* and *Chloromyxum thompsoni* n. sp. from the gall bladder of *Ictiobus bubalus*.

2. Atypical spore formation is recorded in *Chloromyxum opladeli*, and briefly discussed.

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RESEARCH NOTES

A FURTHER NOTE ON LIFE HISTORY EXPERIMENTS WITH *CRYPTOCOTYLE LINGUA* (CREPLIN, 1825)

During the last five years a series of life history studies on heterophyid trematodes have proved unsuccessful owing to the fact that various animals used as final hosts have again and again proved immune to infection.

In the case of *Cryptocotyle lingua* the flukes had failed to develop in laboratory reared white rats, domestic ducks and black-headed gulls, *Larus ridibundus* L. (Rothschild, 1939, Novitat. Zool. Tring, **41**: 178–180), although successful infections had eventually been obtained in two gulls which had been deprived of vitamins in their diet. The long series of worms thus obtained all displayed low egg production. The maximum number of eggs in any fluke was about 30, and the egg shells themselves were very thin. It was suggested at the time that this limited egg production might be accounted for by the fact that the black-headed gull was not a natural host for this species of trematode.

A different explanation, however, seemed worth testing, namely that the metacercariae obtained from certain laboratory infected fish were neither as robust nor as viable as specimens obtained from the same species of fish under natural conditions.

Two gulls were accordingly fed gobies, *Gobius ruthensparri* (Euphras.), which had acquired their infections in the wild. The results obtained certainly seem to confirm this hypothesis.

Eggs began to appear in the feces six days after feeding cysts. When the flukes were recovered from the intestines of the bird, when they were killed three weeks later, almost all proved to be larger than the largest of the specimens obtained from the gulls fed with experimentally infected fish. Moreover, the number of eggs per fluke was far greater than that found in the specimens from the original experiments. Measurements and egg counts of five preserved specimens chosen at random from each group are given below.

	Adult worms obtained after feeding gulls with gobies infected in the wild		Adult worms obtained after feeding gulls with gobies infected in the laboratory	
	Size in microns (maximum length and width)	No. of eggs per specimen	Size in microns (maximum length and width)	No. of eggs per specimen
	1925 × 495	145	1530 × 525	6
	1650 × 525	36	1320 × 525	12
	1305 × 555	191	1140 × 360	24
	1275 × 646	222	1050 × 420	15
	1200 × 660	89	930 × 345	13
Mean:	1471 × 576	137	1194 × 435	14

The most striking feature, however, is the relatively thicker egg-shell found in the first group—the eggs being consequently a rich dark brown in color instead of a deep yellow. This gives a false impression of greater size.

The metacercariae, when removed from the cysts of naturally infected gobies, do not appear to differ morphologically from those which develop in experimentally infected fish. Some specimens, however, are very active, moving about vigorously between slide and cover glass. In these specimens the various organs seem more sharply differentiated, and the cyst wall is more resistant. This active type was not found in the artificially infected gobies, which were all of the sluggish variety, barely showing more than feeble contractions when released from the cyst. On the other hand, in naturally infected fish I have found both sluggish and active metacercariae measuring approximately the same size in the same individual fish in a similar location along the fin rays. On the whole it seems reasonable to suppose that, although apparently quite healthy in the aquarium, few if any fish enjoy such perfect health in captivity as they do in natural conditions. As in the case of cer-

tain snails and their cercariae (Rothschild, 1938, Novitat. Zool. Tring, **41**: 84–102), this poor condition of the host is duly reflected in the lack of vitality and general physiological state of the parasites. This might well account for the frequent failure of the metacercariae to develop and establish themselves in an apparently suitable final host in the laboratory. Whatever the fundamental cause may be, the adult flukes, which were obtained from black-headed gulls fed with naturally infected gobies, are unquestionably more normal specimens and agree more closely with published descriptions of *C. lingua* than those recovered after feeding with metacercariae obtained experimentally.—Miriam Rothschild, *Ashton, Peterborough, England.*

NEW PARASITE RECORDS FROM THE RUFFED GROUSE

In the course of examining a ruffed grouse (*Bonasa umbellus*) from Itasca Park, Minnesota, on August 19, 1940, the author found two species of trematodes hitherto unrecorded from this host.

Two specimens of *Lyperosomum monenteron* Price and McIntosh (1935, Proc. Helm. Soc. Washington **2**: 63–64) were collected in the liver. The specimens agree with the figure in the original description in general appearance and in the peculiar condition of having only one cecum. Measurements of the present specimens in millimeters are given below with the measurements from the original description inserted in parentheses for comparison: body 4.9 to 5.3 (1.9 to 5.2) long; oral sucker 0.234 to 0.277 (0.120 to 0.170) long by 0.213 to 0.284 (0.123 to 0.150) wide; acetabulum 0.213 to 0.284 (0.170 to 0.320) long by 0.249 (0.200 to 0.320) wide; pharynx 0.064 to 0.072 (0.042 to 0.060) long to 0.056 (0.050 to 0.070) wide; ova 0.032 to 0.038 (0.032) long by 0.019 to 0.027 (0.016) wide. The same species was found in the livers of two kingbirds (*Tyrannus tyrannus*) from the same locality.

One specimen of *Echinoparyphium aconiatum* Dietz (1910, Zool. Jahrb. Jena Suppl. **12**: 265–504) was found in the intestine. This species is distinguished from other 37-spined echinostomes by the location of the ventral sucker one-fourth of the body length from the oral sucker and by the shortness of the uterus. The present specimen agrees closely in size with those of Dietz as will be seen in the following measurements given in millimeters. Measurements from the original description are inserted in parentheses for comparison. Body 1.53 (1.62) long by 0.35 (0.26) wide; long spines 0.040 (0.033 to 0.048) long by 0.0112 (0.006 to 0.009) wide; short spines 0.027 (0.028 to 0.045) long by 0.008 (0.0048 to 0.0072) wide; oral sucker 0.085 (0.07 to 0.083) wide; pharynx 0.085 (0.065) long; acetabulum 0.241 (0.22) long; ova 0.1008 to 0.1072 (0.1008 to 0.1032) long by 0.0592 to 0.688 (0.066 to 0.068) wide. Riech (1927, Centr. Bakt. I Abt. Orig. **103**: 279–290) found that cercariae of this species encysted in several species of snails.

The author is indebted to Dr. F. G. Wallace for suggestions in preparing this note.—Nobutaro Ishii, *Lake Itasca Forestry and Biological Station, University of Minnesota.*

A DEVICE FOR ADMINISTERING PARTICULATE MATERIAL TO MICE

In administering parasite cysts or larvae by stomach tube to mice and other small animals one encounters certain difficulties in expelling all of the cysts from either the rubber bulb pipette or the Luer syringe. The simple pump described here was devised in order to provide a dependable means of injecting definite numbers of trematode cysts through a stomach tube. With this pump it is possible to follow the dose of infective material with a "chaser" of water which washes out the tube and both hands are left free for the manipulation of the animal and stomach tube.

A bottle of water with a two-hole rubber stopper is fitted with a delivery tube and an air inlet tube (Fig. 1). The delivery tube is continued by a rubber tube one foot long to the stomach tube which may be of glass or of cellophane (Spur-

lock, 1941, J. Parasitol. **27**: 93). We have found that a piece of surgical stitch tubing cut obliquely at the end is very satisfactory. A small bulb in the glass connection to the stomach tube keeps the cysts concentrated in one place. Midway between the bottle and the stomach tube a rubber tube with the end plugged and long enough to reach the floor is connected to the delivery tube by a glass T. Two small Bunsen valves are inserted, one on either side of the T. The Bunsen valve (Fig. 2) is made by fitting a one-inch piece of a No. 10 soft rubber catheter on the

Fig. 2.

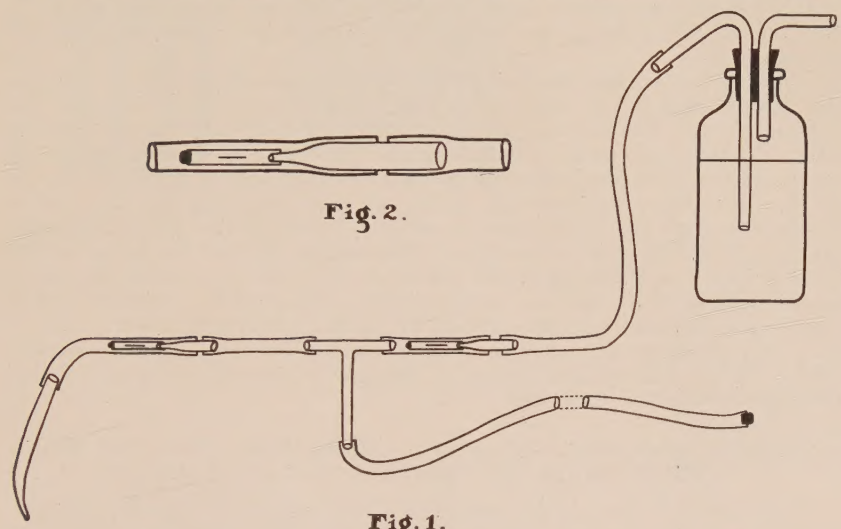

Fig. 1.

tapered end of a piece of glass tubing. The end is plugged and a longitudinal slit is cut with a razor blade. When the entire system is filled with water and all air bubbles are expelled it forms a miniature pump and when one steps on the tube on the floor a few drops of water are ejected from the end. The rubber tube to the left of the second valve is used as a pipette in order to suck up the cysts. Once in the bulb the cysts are locked in place by the valve until pressure is applied to the pump.—F. G. Wallace, *Department of Zoology, University of Minnesota.*

THE DURATION OF HUMAN INFECTION WITH *ENDAMOEBA HISTOLYTICA* AND OTHER INTESTINAL PROTOZOA

A few students in the Vanderbilt University School of Medicine have been observed over a period of years with regard to their intestinal protozoa. Students harboring *Endamoeba histolytica,* who had had no symptoms referable to the infection, were requested not to obtain treatment, so that the duration of their infection could be determined. Two stool examinations were performed in October of the first academic year, another during May of the second year, and thereafter at irregular intervals. The following report concerns nine students who harbored *E. histolytica.*

In two students observed for four and a half years, stool specimens were positive each time they were examined. In two observed for three and a half years and in three observed for one and a half years, the same results were obtained. Of the other two students, one was positive on the first examination and negative on the second examination one and a half years later and continued to be negative up to four and a half years. The other was positive on the first examination and negative on the second examination one and a half years later. He was not observed thereafter.

Complement fixation tests were performed two or more times on seven of these students in our laboratory, using an alcoholic extract of washed motile amoebae as antigen, and an antisheep hemolytic system. All were negative. Colonel Charles F. Craig performed one complement fixation test on six of these, using an anti-human hemolytic system, and obtained ++++ reactions in two, +++ reactions in two, and ± reactions in two. One of Colonel Craig's +++ reactions was obtained in a student whose stool specimen had been negative for *E. histolytica* on several occasions for at least two months before the blood was drawn for the test. None of these students developed symptoms referable to amoebiasis during the period of observation.

Observations of other intestinal protozoa in the same students were as follows: One student observed for four and a half years showed *E. coli, E. nana* and *Iodamoeba* on the first examination and one and a half years later, and *Iodamoeba* after four and a half years. The student who was observed for four and a half years but showed *E. histolytica* only on the first examination also showed *E. coli* and *E. nana* on this examination but not on subsequent examinations. One student observed for three and a half years showed *E. coli* only on the first examination, but showed *Giardia* on each examination. One student observed for one and a half years showed *E. coli* throughout this period, and *E. nana* was found at the end of one and a half years but not on the original examinations. Another student observed for one and a half years showed *E. coli* throughout this period.

Although this series is small, it is recorded at this time because of the impossibility of further observations.—HENRY E. MELENEY, *Vanderbilt University School of Medicine, Nashville, Tennessee.*

SIGMODON HISPIDUS HISPIDUS, A NEW HOST FOR THE STROBILOCERCUS OF *TAENIA TAENIAEFORMIS*

We recently received at this laboratory a male cotton rat, *Sigmodon hispidus hispidus*, from the Michigan Department of Health. This animal was from their breeding colony maintained for investigations on poliomyelitis. It was one of the original stock, having been trapped in South Carolina in February, 1940. The rat had been partially eaten, but examination of the liver revealed the presence of eleven strobilocerci of *Taenia taeniaeformis*. They were from 7 to 10 mm in diameter fixed. The larvae removed from the cysts were from 84 to 135 mm in length with a mean of 114 mm. There were from 32 to 38 hooklets on the rostellum, the large hooklets averaging 316 microns in length and the small hooklets 238 microns.

To the best of our knowledge this is the first record of the strobilocercus of *Taenia taeniaeformis* from the cotton rat.—PHILIP A. HAWKINS, *Department of Bacteriology and Hygiene, Michigan State College.*

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